Blood Volume
and
Contractile Protein in Heart Muscle

Selected Papers
from

SECOND WORLD CONGRESS OF CARDIOLOGY AND TWENTY-SEVENTH ANNUAL SCIENTIFIC SESSIONS OF THE AMERICAN HEART ASSOCIATION

Held in Washington, D.C.

World Trends in Cardiology

I: Cardiovascular Epidemiology
II: Cardiovascular Surgery
III. Blood Volume and Contractile Protein in Heart Muscle
IV. Cardiovascular Diagnosis and Therapy
V. Instrumental Methods in Cardiac Diagnosis

World Trends In Cardiology: III

Blood Volume and Contractile Protein in Heart Muscle

EDITED BY

ARTHUR S. CAIN, JR., M.D.

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BLOOD VOLUME AND CONTRACTILE PROTEIN IN HEART MUSCLE

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FOREWORD

More and more in the past few decades have the clinical scientist and the basic scientist been drawn together. A mutuality of interest has developed, and, in research, what amounts almost to a mutual dependency has led to increased understanding and a reciprocal appreciation, each of the other, within the fraternity of biological scientists.

Certainly this relationship is no more apparent or important than in the field of circulatory function and disease. The underlying importance of a thorough understanding of the normal physiological control and biochemical activity of the circulatory system, if one is to correct its malfunctions, is unchallenged.

In recognition of this concept, there was presented at the Second World Congress of Cardiology a special group of papers devoted to the physiology of blood volume and the control of the peripheral vascular bed and to the physiology and chemistry of the contractile proteins of the heart.

These papers by distinguished scientists in the field are presented in this volume from the scientific program of the Second World Congress of Cardiology and the Twenty-seventh Annual Scientific Sessions of the American Heart Association.

A. S. C.

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PART I

Blood Volume

1

Control of the Peripheral Circulation by the Walls of the Blood Vessels

THE GEORGE E. BROWN MEMORIAL LECTURE

Alan C. Burton, London, Canada

DR. GEORGE E. BURCH *of New Orleans introduced the George E. Brown Memorial Lecture, and the lecturer, with the following remarks:*

The lecture that is to be given now was named in honor of Dr. George Brown, who was in charge of the Section on Peripheral Circulation at the Mayo Clinic. It was Dr. Brown who was responsible for the organization of the Section. He was to be its first chairman, but, unfortunately, expired just before he had the opportunity of presiding over the first meeting.

As a result of Dr. Brown's contributions to science—particularly in the field of the peripheral blood vessels—and his interest in the American Heart Association and the organization of this Section, the George Brown Lecture was organized several years ago. It has been the privilege of the chairmen each year to select a speaker for the meeting of the Section. This year, we are particularly fortunate in having as our guest speaker Dr. Alan C. Burton, who is Professor of Biophysics at Western Ontario University, in Canada Dr. Burton, I am sure, is well known to all of you, but I should like to make a few comments.

Dr. Burton has been interested in many phases of biophysics and has had a considerable interest in the biologic aspects of biophysics. He has made important contributions in the problems of thermoregulation He has worked out many methods for calculation of heat exchange, from the surface of the body of man, under various environmental conditions. His contributions during the last war, for example, in making recommendations concerning clothing and protection of troops from various types of weather conditions, were particularly outstanding. He has made important contributions in the peripheral circulation, particularly in the circulation in the digits, and has continued, at the same time, to show a great deal of interest in the general science of medicine, physics, and teaching. He has been responsible for the training of a number of our active investigators, whom we find throughout this country and Canada today.

It gives me great pleasure to be able to introduce to you Dr. Burton Dr. Burton will speak on *Control of the Peripheral Circulation by the Walls of the Blood Vessels.*

DR. ALAN C BURTON: I count it a very great honour to have been asked by the Chairman of the Circulation Section of the American Heart Association to deliver the annual George Brown Lecture. I accepted the invitation and supplied a title with too much haste, and ever since have been worried about my ability to do justice to the occasion. Finally, I decided that I must ask you to give a most liberal interpretation to the title that I supplied.

My sense of inadequacy has been greatly increased after reading, in preparation for this moment, many of the lectures given by my distinguished predecessors. Dr. George Brown was responsible for the formation of this Section for the study of peripheral circulation, and his lifetime interest was in the clinical investigation of peripheral vascular disease. It seems to be the duty of a George Brown lecturer to try to contribute in some way to that field. Here is where my own incompetence is so patent since, as you may know, I am almost completely without clinical experience of any kind, certainly of peripheral vascular disease. I can only hope that your Chairman, in asking

me, wanted the underlying physiology of the control of peripheral circulation to be emphasized, to direct your thoughts toward that body of facts and understanding of the normal functioning that must underlie any intelligent management or treatment of pathologic physiology.

If this be so, I find it easier to justify my standing here. I do have enough contact with my clinical colleagues and their patients with peripheral vascular disease to get the impression that whatever the causes of their disease or the site of the actual pathology, it is with symptoms in fingers and toes that the doctor must usually have immediate concern. So much is the peripheral vascular specialist concerned with fingers and toes that the College of Heraldry might well consent to recognize, as the professional sign outside the office of a peripheral vascular specialist, a shield showing two big toes on a purple background, with three white pills (codeine and aspirin) beneath. It would, no doubt, be described as "two digits, amputated, rampant, and gangrenous, on a field cyanotic"

If, then, I can use this excuse to talk about the physiology of blood flow of the skin, particularly of the digits, I can feel some competence. I think that I was one of the first people, actually, along with Dr. Burch, to measure the blood flow in the fingers,[4] and I think that he and I can be described as the "high priests of digital plethysmography."

The lecture will have some application to the symptoms of peripheral vascular diseases, if not at all to the diseases themselves. *What I have to say about the control of blood flow of* the digits may possibly give my audience, so much better acquainted with peripheral vascular disease, new ideas as to the proper treatment of such disease. Certainly, I hope to impress upon you how much more definite physiological information we need to obtain, by careful research in human physiology, before we can feel confident that our present methods of treatment are logical. I hope, then, you will take this possible application of the physiology as your task.

To one who is largely an onlooker in clinical investigation and treatment of peripheral vascular disease, the scene is one

of great confusion. It is easy to find experts who differ diametrically in their views. After Dr. Gaskell and I had completed some studies of the effect of posture on blood flow of the toes,[10] it occurred to us that there might be some application of what we had found to treatment of Buerger's disease, so I asked our Professor of Surgery this question: "With a patient in bed with Buerger's disease, do you advocate keeping the leg up, level, or down?" After a little thought, his reply was: "Well, Alan, there are three schools of thought."

Then, again, I meet most enthusiastic advocates of sympathectomy, and some who strongly disapprove. I have heard that the application of positive pressure is beneficial, and also that it should be negative pressure, and alternate positive and negative pressures. In frostbite, which is a peripheral vascular accident, if not a disease, large quantities of heparin have been injected, though experimental research on animals has reached no conclusive evidence that it helps. Some rapidly rewarm frostbitten digits; others pack them in ice for weeks. There are enthusiasts for exercise in phlebitis; others use complete rest.

When we hear of a group of people rushing in diverse directions, we usually come to one of two conclusions; either they are in a panic and unable to think clearly, or else they are in the dark, in surroundings about which they have little knowledge. I will not insult you by choosing the first alternative, so I conclude that what is most needed in the field of peripheral vascular disease is more light, more definite, quantitative information as to the peripheral blood flow and how it is affected by heat, cold, blood pressure, nervous control, reflexes, posture, and so on.

I am not ignorant of the considerable body of research done already by many of my audience on these points over a period of many years. I think that the difficulty in interpreting and applying that research has been in the diversity of experimental methods used to estimate the circulation and, perhaps, in some cases, in a failure to analyze just what quantity was being measured.

Let me illustrate by citing the use of the "circulation time"

of a limb, which nowadays is relatively easily measured, even in patients. It does not seem to be generally realized that this depends just as much on the volume of blood between the site of injection and the site of detection as it does on the volume of blood flow per minute. In fact, the relation is:

$$\text{Circulation time (sec.)} = \frac{\text{Vol. (ml.) between A and B}}{\text{Vol. flow (ml./sec.) between A and B}}$$

Now, since physiologic changes of blood flow usually occur because the vessels narrow or widen, a change in blood flow, brought about by the nerves or by posture or whatever else, is almost certainly accompanied by a simultaneous change in the volume of the vascular bed. The circulation time, when the blood flow decreases, may even in some circumstances actually become shorter because of a simultaneous decrease in volume of the bed. Yet observations on changes in circulation time have often been assumed to give clear guidance as to the direction of change of the volume flow

To turn to indices of blood flow that we applied more easily in the clinic, Sir Thomas Lewis[15] showed that the color of the skin depends on the amount and on the state of reduction of hemoglobin in the subpapillary venous plexus. Again, there is a rather remote connection with the amount of blood flow I would remind you that the rosy cheeks of our children when they are out in the cold do not indicate a hyperemia at all, but, rather the tenacious retention of oxygen by hemoglobin when its temperature is lowered. There are also valid objections to the interpretation of changes in skin temperature, where the blood volume of a limb is altered as well as the flow.

I would suggest that even changes in pain and discomfort of the patient himself, when a given procedure is tried, may, in peripheral vascular disease, be a fallacious guide *Are we certain* that a patient suffering from Buerger's disease should not have his leg elevated in bed, because he may report that he is more comfortable with the leg dependent? May not the relief of pain be an indication of failure of nerves to conduct when ischemia is complete, and the return of pain be the result of an increased

circulation? I have lately been the subject in experiments when the blood flow of the hand is completely occluded for up to 25 minutes by a pressure cuff, and I can testify that the pain when circulation is restored is far more violent than during the occlusion, and, of course, everyone knows this is true of frostbite.

It seems, then, that the important starting point is the decision as to what is the relevant variable to measure, and then we should adopt the method that measures this variable as unequivocally as possible. I submit that the relevant quantity is the volume rate of blood flow to the tissues rather than the linear velocity of flow, the circulation time, or other quantity. I will agree with Dr. Hugh Montgomery[16] that it is the supply of oxygen rather than the blood flow which is of most importance, and I am delighted that he is successfully measuring oxygen tensions in peripheral tissues by the polarograph. The new clearance methods (originally due to Kety) in which the rate of washing out from the tissues of radioactive or other indicator substances is measured,[17] should also prove of great value in the study of peripheral vascular disease. In many cases, however, the measurement of the volume blood flow, for which there has been so long the classical method of the venous-occlusion plethysmograph, will tell us definitely what has happened to the nutrition of the tissues, since a change in the blood-tissue barrier is not in question.

I will, therefore, illustrate what progress can be made in definite knowledge of the effect of different factors on the peripheral circulation, and how much more needs to be done, by outlining the results of research on the blood flow of the fingers and toes

DIGITAL BLOOD FLOW

My choice of the fingers to measure blood flow was not, originally, because of specific interest in the digits, but because, in the fingers, one has a total blood flow which is, except for an almost negligible bone-flow, entirely the blood flow of the skin. The classical methods of measuring flow by the venous-occlusion plethysmograph—devised by Hewlett but originally sug-

gested by Brody and Russell and so much used by Lewis and his pupils—have long been applied to the forearm and to the hand, notably by Professor Henry Barcroft and his students [1] In the forearm, however, we have, perhaps, 70 to 80 per cent of the flow going to muscle, the rest to skin, with a small part to bone In the hand, skin flow and muscle flow are more nearly equal. Since we now know how different are the physiologic control and the reactions of the blood vessels of muscle from those of the skin, it is no easy matter to interpret changes in total flow in the forearm or hand in terms of what has happened in either muscle or skin. I recommend to your reading the excellent monograph recently published by Barcroft and Swan [1]

A study of the blood flow of the finger or toe, however, gives us direct information as to the range and fluctuations, and the means of control of the blood flow of the skin, at least of skin of this particular site. While we have no right to assume that the skin of the digits is representative of all skin circulation—indeed, I will point out some marked differences from skin elsewhere—we do not have a second, very diverse tissue complicating the picture.

Application of the classical method turns out to be very simple in the fingers. I do not think I need to tell you the details of the apparatus which has been modified by many. Toes are a bit more troublesome, as their geometry is far from ideal for our purpose, but the big toe is usually adapted to the method.

Figure 1 shows how the blood flow is calculable from the records from the initial rate of increase of volume at the moment when the venous outflow of the digit is prevented by the inflation of the occluding cuff. The period of occlusion by the cuff around the first phalanx of the finger is indicated by the solid line along the baseline. The shape of these curves is so well known to most of you that I do not need to describe them. Dr. Burch, in his recent monograph,[2] has submitted the method to rigorous scientific criticism, from which, I feel, it has emerged as still the best method for measuring blood flow in the human limbs and digits. It gives us flow directly in millilitres per minute.

Some remarkable features of the blood flow of the digits emerged from the early work. These are, first, the remarkable range of flow encountered, and, second, the remarkable degree of fluctuation of flow from moment to moment.

As to the range, this is from a minimum of about 1 ml./min./100 ml. of tissue in intense vasoconstriction to a maximum of

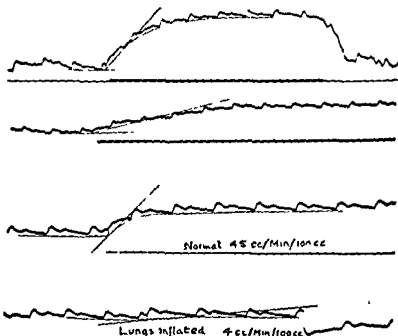


Fig. 1

at least one hundred times this value. Why this extraordinary range of blood flow in the fingers?—especially when we consider that the needs of skin for oxygen and, thus, for blood flow are so modest and so relatively constant (though, as with all metabolic processes, it must change with the temperature of the skin.) From values in the literature on the oxygen consumption of skin, one may calculate the minimum requirement of circulation (very roughly, it is true) as being about 0.8 ml./min./

100 ml. of tissue, and a guess would be that the maximum requirement would not be more than two or three times this.

The tremendous range of flow found in the finger, then, cannot *represent the response to the needs of this tissue for blood* flow. Grant,[12] many years ago, showed us that, instead, the range and great magnitude of peripheral flow in the rabbit ear serves the purpose of temperature regulation of the whole animal, and in man this is one of the major mechanisms of increasing the heat loss of the body when this is required to keep the heat balance. The function of control of digital flow for this purpose has been verified by the researches of Taylor and myself[6] and by many other workers.

I like to persuade undergraduate classes to define for themselves the function of the circulation. Their final definition usually runs like this: "The function of the circulation is to convey oxygen, foodstuffs, and heat, and to remove the products of metabolism and heat, to each tissue in accordance with its needs."

In the circulation of the digits and probably of much of the skin, we have a special case. Here, the control of the blood *flow is not in accordance with the needs of that particular tissue*, the skin, but with the needs of the whole organism. In the most intense physiologic state of vasoconstriction, the minimal blood flow would appear to be adequate to maintain the metabolism of skin. Indeed, men have continuously maintained intense vasoconstriction of fingers and toes for long periods, as in many climatic experiments, without any sign of impending death and gangrene of the tissues.

From this, I draw two conclusions: first, that the regulation of the blood flow of the digits is unrelated to the needs of their tissues, and, consequently, the digits do not possess the autonomy of other tissues such as muscle, where the blood flow is regulated according to the local demand for oxygen. The increased need of muscle in exercise is met not only by integrative mechanisms of the whole organism, as by the dilating effect of circulatory adrenalin and of sympathetic discharges, but also by the local *autonomous regulation, which is known as reactive hyperemia.*

It is only recently that I realized that I, with others, had too complacently accepted reactive hyperemia as a general principle of local control of the circulation of all tissues[16] Actually, it has been clearly demonstrated and studied only for muscle. We have reason to believe that in the skin, reactive hyperemia is of much less significance and is easily abolished in certain physiologic circumstances.[14] Again, when I search the literature, I find that reactive hyperemia does not occur at all for the mesenteric circulation, nor for the kidney, nor the liver. It is time we ceased from teaching medical students that this is a broad

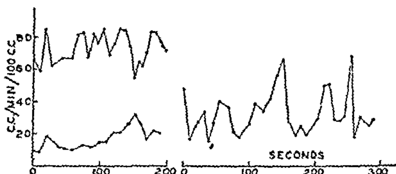


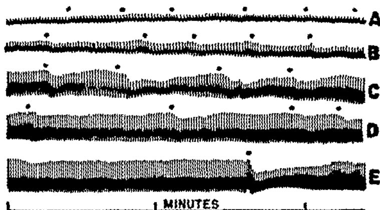
Fig 2

principle of physiology, however attractive this may be teleologically It seems likely that this relatively small autonomy of control, and the greater subservience of the skin circulation to the needs of the whole body, make the skin peculiarly susceptible to damage by ischemia when normal physiology is disturbed, as in peripheral vascular disease

The second conclusion I draw from the analysis of the range of digital blood flow and the very low metabolic needs of skin is that when death of skin occurs, as in gangrene, the blood flow must not merely be low, but actually less than that found in intense vasoconstriction. This means that gangrene of the toes or fingers indicates practically a zero blood flow. I will return to this point later.

Let us turn to fluctuations of digital flow and the nervous control. In addition to its striking range of values, the digital

blood flow is remarkable for its fluctuation from moment to moment. It is easily possible to take a long series of blood flow determinations on the finger at six-second intervals. The statistical standard deviation or coefficient of variation of the successive flows can be used as a measure of the fluctuation. For the middle range of blood flow, the coefficient of variation amounts



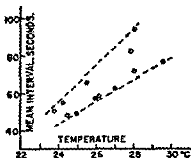


Fig. 3

to plus or minus 40 per cent. The fluctuation is less for states of vasoconstriction and for states of full vasodilation In Fig 2 you see the remarkable fluctuation from measurement to measurement.

Continuous recording of the volume or volume pulsation of fingers and toes reveals the nature of this continual fluctuation (Fig. 3). There are periodic vasoconstrictions, simultaneous in

all the digits, and accompanied by transitory increases in heart rate and, as we were able to show by special methods, by increases of a few millimeters of mercury in the blood pressure. From *A* to *E* are records taken with the subject cold in *A* to

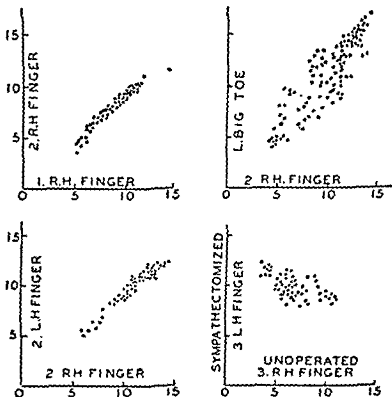


Fig. 4

very warm in *E*. The frequency with which these vasoconstrictions occur is governed normally by the needs of the body for temperature regulation. This is shown on the graph on the left-hand side. These periodic vasoconstrictions represent mass discharges of the sympathetic vasoconstrictor nerves, mass discharges because the impulses reach all the digits and heart also,

as shown by its periodic accelerations, and because the general vasoconstriction is so widespread that a detectable rise of blood pressure results.

Figure 4 shows the correlation of the simultaneous size of pulsation in the fingers and toes, and how remarkably good this correlation is. Every time one has a vasoconstriction or vasodilation in the finger, there is a simultaneous change in the toe. On the bottom right-hand side, the graph is for a patient who had a sympathectomy of one arm, and there you see the correlation is opposite This proves that these simultaneous fluctuations were mediated by the sympathetic nervous system.

CHEMICAL VS. NERVOUS CONTROL OF DIGITAL CIRCULATION

Presumably, the blood vessels of the digits are dilated by general chemical dilator agents, as are all other vessels. Yet, we found that when amyl nitrate, a general vasodilator drug, is inhaled, producing a profound dilation of the vessels of the skin of the blush area, the blood flow of the fingers falls to zero. Evidently, the discharges in the vasoconstrictor nerves elicited by the fall of blood pressure, through the aortic-arch, carotid-sinus buffer mechanism, *completely outweigh the direct* chemical effects in the digital vessels. We learn again how completely the circulation of the extremities is the servant of the needs of the whole organism The dominance of neural vasoconstrictor *control of these vessels is complete.*

NEUROHUMORAL CONTROL OF THE CIRCULATION

We now know, thanks to the excellent work of the Swedish workers such as Von Euler, Folkow, Zotterman, and of Professor Burn at Oxford,[8, 19, 20] a great deal about how the sympathetic constrictor discharges produce the contraction of the smooth muscle of the arterioles which is so effective in controlling the blood flow. This is through the mediation of the vascular hormone, noradrenalin, which is liberated at the nerve endings. Also, recent research is showing how much more is involved. The presence of the enzyme, amine-oxidase, which rapidly

destroys noradrenalin at the site of its production, has been shown in the walls of arteries and veins, and in some cases the concentration of this enzyme has been measured. The interrelations seem exactly analogous to those of acetylcholine and cholinesterase, the mediator of the parasympathetic system, about which, because of the classical work of Sir Henry Dale and his colleagues, we have known much longer. The analogy goes even further, in that, just as we know of antagonists of the destructive enzyme, cholinesterase, such as eserine, we now know that ephedrine acts as a constrictor, not directly upon the smooth muscle of vessels but by competitive inhibition of the amine-oxidase.

This new knowledge of sympathetic neurohumoral mediators and the enzymes that keep them in balance should open up an entirely new field of exploration in peripheral vascular research. Who knows that certain peripheral vascular diseases may not, in part, be due to a lack of balance of the liberation of noradrenalin by nerve endings and of the enzymes that keep it in check?

It is to be hoped that research will be actively pursued in the concentration of amine-oxidase in the small blood vessels, and, since assays can be made on autopsy material,[19] there is no insuperable difficulty. Some very recent experiments, physiologic rather than biochemical, which I will mention to you later, strongly suggest to us that such research would pay large dividends for the study of disease.

On the cholinergic side of the picture, an important advance has recently been made by the same Ronald Grant who pioneered in the study of the minute vessels of the skin.[18] Those concerned with sympathectomy have long been puzzled to account for the regaining of almost normal tone, but not of reactivity, of the blood vessels of a limb a few days after complete sympathectomy. The theory that this reflected an increased sensitivity to circulating adrenalin, the so-called "denervation sensitivity," was never satisfactory. Grant has now shown that there is always present in the blood emerging from peripheral vascular beds, a dilator agent which most probably is

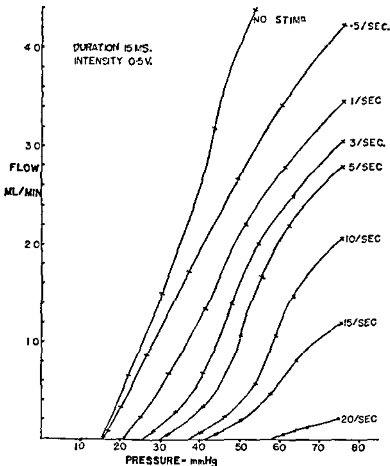


Fig. 5

acetylcholine, liberated from sympathetic dilator nerve endings. The normal tone of the vessels is the result of a balance between the constrictor influence of the noradrenalin and the dilator influence of this acetylcholine. Sympathectomy eventually stops the liberation of both these antagonistic hormones, but, as Grant has shown, while the constrictor agent disappears almost

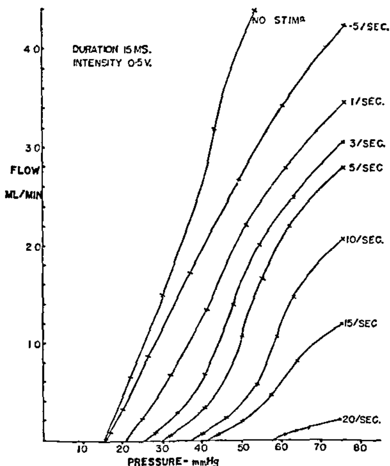


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immediately, the dilator agent persists for a few days. Hence, there is an uncompensated dilation in the first day or two, followed by a return to "pseudo-normal" tone when acetylcholine also has disappeared. Cholinesterase in the tissue also disappears, but later than the acetylcholine. Again, new fields for future research of importance to peripheral vascular diseases are being opened up.

THE RELATION OF FLOW TO BLOOD PRESSURE

If we ever are to understand the physiology of peripheral vascular disease, we must understand the underlying hemodynamics, particularly the relation between the driving force, which is the difference in pressure between artery and vein, and the flow through vascular beds that results. The underlying physical analysis of the equilibrium of the wall of the blood vessels, under the opposing forces of the tension in the wall due to elastic tissue and to smooth muscle has been discussed in many places by myself and by co-workers,[5] and more discussion would be inappropriate in this lecture, so I will confine myself to describing the curves of flow vs. pressure that we have obtained from study of the rabbit ear, of the human forearm, and of the fingers. A figure from Dr. Girling's work on the rabbit ear,[11] illustrates that complicated relation best (Fig. 5).

When vessels are dilated, with very little vasomotor tone, the relation is practically a straight line, which would correspond to the classical ideas of the flow-pressure relation. The stimulus frequency referred to in the figure is that of the stimulation of the cervical sympathetic ganglion, causing greater constriction in the rabbit ear. But, as vasomotor tone increases, the curves become sigmoid and finally concave to the pressure axis. Moreover, contrary to classical ideas but explained easily on the basis of physical analysis of the equilibrium, the point where the curves cross the pressure axis moves to higher pressures. This pressure, below which there is no flow at all, we call the "critical closing pressure," and it is a useful measure of the tension in the wall of the arterioles. We have now succeeded not only in explaining the existence of critical closing pressures in terms

of simple physics, but also in explaining the changing shape of all these curves in terms of the distensibility of the vessels which are responsible for the resistance to flow.

Mr. Yamada in our laboratory has ingeniously developed methods for at least estimating the flow-pressure curves in the human forearm and in the fingers, and these follow the same pattern as for the rabbit ear (Fig. 6).

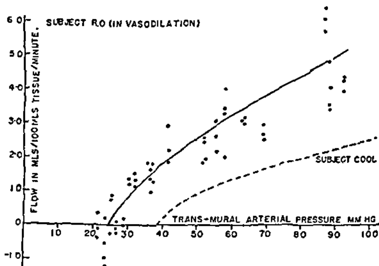


Fig. 6

Critical closing pressures in the arm and in the finger range from a minimum of about 10 mm Hg. when the vessels are fully dilated, with the subjects very hot, to 70 mm. Hg. when they are very cold and constricted [7] In hypertensives, we find still higher values. However, in vasomotor spasm, the critical closing pressure can rise to much greater levels, in fact, we think of spasm of a blood vessel as meaning simply that its critical closing pressure has risen, by reason of increased tension in the wall, to a level above the available blood pressure. We have verified this completely in animal experiments.

This new knowledge of the relation between pressure and flow in the peripheral circulation, which is so different from the classical concepts, should have two most important results in our thinking. It gives the level of the arterial pressure per se an entirely new importance. While in dilated vessels, fall or rise of blood pressure will produce a change in flow which is roughly proportional, in vessels under high vasomotor tone, it will produce an effect on flow out of all proportion to the change in pressure. A change of a few millimeters of mercury in the driving pressure, in vessels which are close to the point of critical closure, may change the flow from nothing at all to a very considerable flow Again, Girling's curves can be interpreted to show that the effectiveness in reducing flow of a given activity in the sympathetic is greatly increased by a fall of blood pressure—a fact of great importance in hypotensive shock.

This great dependence upon blood pressure of vessels under vasomotor tone has explained, for me, two very puzzling observations in connection with peripheral vascular disease. Professor Dible of Oxford[9] has injected, with radiopaque material, the amputated limbs of patients with chronic obstruction of the main leg arteries, where the toes had been gangrenous (Fig. 7). He found that the vessels of the foot were very completely injected, possibly more than in the normal, through the long, thin collateral channels that had developed. The arteries of the foot, then, were receiving plenty of blood. Why was there gangrene in the toes, especially in view of the fact that he could find no trace of diseased processes in the digital vessels? On classical views of the pressure-flow relation, there should have been plenty of oxygen supplied to the toes. With our new knowledge of this relation, we can see that it is not sufficient to supply blood, blood must be supplied at a sufficient arterial pressure. The collaterals that have developed have much more resistance than the original arteries, and there is loss of pressure down them. The arterioles of the toes evidently have suffered a "critical closure."

The second puzzle now elucidated, at least for us, is the fol-



Fig 7

lowing You have all heard much from those who advocate the use of sympathetic blocking agents in the treatment of peripheral vascular disease but I understand that others feel the results have been sometimes most disappointing If the trouble was due to excessive vasoconstrictor tone of the peripheral vessels, why

should not adrenergic blockade have favorable results in all cases? We think we stumbled upon an answer when we were investigating the effects of posture on the flow and pulsation in the digits

Critical closure can be produced by reducing the absolute hydrostatic pressure in all the vessels simply by raising the limb, as Dr. Gaskell and I have shown [10] In Fig 8, *A* and *B* are the simultaneous records of the pulsation in the two big toes, both legs being horizontal, and you will note how the changes in

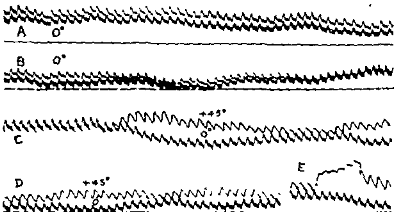


Fig 8

volume and in pulsation coincide in the two toes They fluctuate together like one record. This agrees with Fig 5, where the spontaneous fluctuations of volume pulsation and of flow were simultaneous and completely correlated in all the digits In the lower records, we simply raised one leg at an angle of 45° above the horizontal The fluctuations became not identical but reciprocal. When a spontaneous vasoconstriction shows itself in the record of the horizontal limb, the volume pulsation in the elevated toe becomes greater Moreover, a fortunately timed flow measurement, at *E*, showed that not only pulsation but also blood flow was increasing in the raised limb at the time when there was a general vasoconstriction

Now, no one could persuade me that the mass discharge, going to all the other digits, was not simultaneously reaching the vessels of the elevated toe. How, then, was this paradoxical increase in flow produced? The only explanation seems to be that the vessels of the elevated toe, by reason of the reduced hydrostatic pressure, were in the critical condition where the slight rise of blood pressure, known to accompany these general vasoconstrictions, had so great an effect on flow that it overshadowed the simultaneous increase in tone of these vessels.

Now, we see how, in vessels which are under high vasomotor tone and consequently close to this condition of critical closure, a blocking agent, while it increases the flow elsewhere, may actually decrease the flow in the affected digit by reason of the accompanying drop of blood pressure As somebody has said, "We would use the blocking agents in peripheral vascular disease with more confidence if the drug companies would supply us with a specific blocking agent for the left big toe, for the right index finger, and so on."

THE EFFECTS OF POSTURE

The size of the blood vessels which control the flow, that is, the arterioles, depends, as with other vessels, upon the hydrostatic pressure within, or, more accurately, on the difference of pressure inside and outside the vessel. (We call this the "transmural pressure") We therefore expected that raising the limb would decrease the flow in a digit, because the hydrostatic pressure in all vessels is reduced This was what Gaskell and I found,[10] in experiments on a tilt table where one leg could be raised and the other kept level as a control. What was quite unexpected was our finding that there was a maximum at or about heart level, and lowering the limb also decreased the flow markedly (Fig 9). This was an effect on the test leg only, not due to a change of central blood pressure, as shown by the lack of change in the toe of the control, horizontal, leg. No explanation of this reduction of flow in terms of physics appears possible, and in the records there was striking evidence that the distention of the veins or smaller venous vessels was producing a

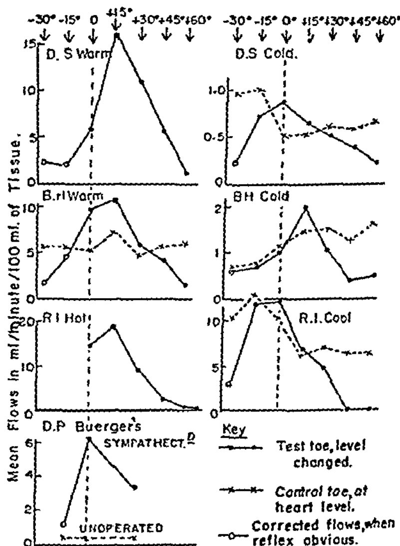

-30° -15° 0 +15° +30° +45° +60°
-30° -15° 0° +15° +30° +45° +60°
D. S Warm
D.S Cold.
B.rl Warm
BH Cold
R I Hot
R.I. Cool
D.P Buerger's
SYMPATHECT.D
UNOPERATED
Mean Flows in ml/minute/100 ml. of Tissue.
Key
Test toe, level changed.
Control toe, at heart level.
Corrected flows, when reflex obvious.

Fig. 9

local constriction of the arterioles. The evidence for this "venivasomotor" reflex was present in a leg in which the sympathetics had been cut ten days previously, so we postulated that it was mediated by some local nervous connection between the venous and the arteriolar sites of a peripheral vascular bed. This could be an axon reflex or possibly involve a plexus ganglion. It was clear that venous distention was the eliciting factor, for the reduction of flow occurs whether we fill the veins by lowering them, by raising the heart, or by applying negative pressure to the tissues.

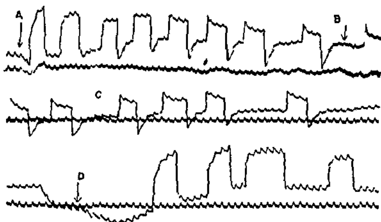


Fig. 10

In Fig. 10, before the record was taken, the leg was level, and the flow records were normal. At *A*, the man sat up, so that his heart was raised above the level of the toe vessels, and at once you see the beginning of the change in this record, such that during the occlusion the volume decreases, and when one removes the occluding pressure used in the measurement (venous occlusion pressure) the volume drops below the line, showing that there has been a shrinkage of the volume of the toe. Then it recovers. At *B*, the subject lay down again, but this peculiar effect, which is evidence of "venivasomotor" reflex, persisted through *C* until, at *D*, we momentarily raised the leg and drained

the vein and put the leg horizontal again. Only then did the effect disappear.

Figure 11 shows the effects of tissue pressure on the finger, studied by Mr. Yamada. This is a schematic graph based on the average of many experiments. Once again, you see that not only does increasing the tissue pressure decrease the flow—that is expected (since it reduces the transmural pressure), but the un-

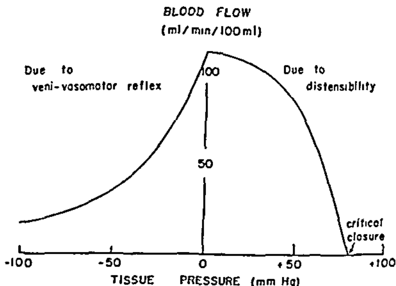


Fig. 11

expected happens, again, that when negative pressure is applied, which fills the veins, there is a decrease of flow.

I have no time to go into details of the experiments that convinced us that we must be dealing with a peripheral vascular reflex, hitherto rather neglected, that could play an important part in peripheral vascular disorders. I am sure, however, that many of my colleagues, whose opinion I respect, remained unconvinced and prefer some other explanation of the phenomena we have demonstrated. Vascular surgeons, on the other hand, have long thought that there must be some nervous influence of

the veins on the vasomotor control of the arterial side, for in phlebitis, I understand local spasm of arteries is often seen when the pathology is in quite distant venous vessels Dr. Ochsner, Dr. Burch, and Dr. de Bakey[8] have discussed this in several papers.

Recently, quite by what Cannon called "serendipity," or the unexpected dividends of research when the experiment was designed to look for something quite different, Mr Yamada, Dr. Leach, and I came across new evidence of the neurohumoral agents that may be concerned. This discovery was that the evidence of vasoconstriction upon venous distention was much more marked in a digit after complete arrest of the circulation, than it was with the blood flowing.

We have made exploratory experiments along this line, and our speculative interpretation is that we can obtain, by plethysmographic methods, some measure of the amount of a constrictor substance, presumably noradrenalin, released in response to venous distension, and its destruction presumably by amine-oxidases The time of recovery of the vessels from the vasoconstriction so elicited is markedly affected by the time the complete circulatory arrest has been applied. In order to test our hypothesis of noradrenalin liberation, we have studied—so far in only one subject—the effect of a large dose of ephedrine on the phenomena, for this drug should inhibit the amine-oxidases. Our expectations were met. A dose of 45 mg gave definite evidence that the recovery from the "reflex" vasoconstriction was slowed, and might never be complete. The blood pressure rose from 115/70 to 132/90, but was back to normal in an hour. A dose only 25 per cent greater gave much more dramatic results. The evidence of vasoconstriction was still greater, and now the recovery was never complete. It was as though the amine-oxidases still unoccupied by the ephedrine were insufficient to deal with all the noradrenalin released The blood pressure rose to 158/100 and remained with great fluctuation at that level for three or four hours, in fact, until I took barbiturates to correct it.

As the subject, though I had no ill effects whatever, I was greatly impressed with the peculiar dangers of a drug which interferes with a protective enzyme of the circulation, and how

narrow is the range between safe dosage which leaves some enzyme effective and a potentially dangerous dosage which leaves none. In the latter case, presumably, adrenalin and noradrenalin liberated in exercise or excitement continue to circulate relatively unchecked in their constrictor and other effects. We cannot help wondering if some peripheral vascular conditions and, perhaps, some forms of hypertension might not be connected with abnormalities in the noradrenalin-amine-oxidase balance. We are naturally excited at the possibility of studying the physiology and biochemistry of the vascular hormones by using the finger plethysmograph

My purpose in mentioning this contemporary, undigested, and unverified research is not only to enliven somewhat a rather dull lecture, but to show you how much more research relevant to peripheral vascular disease needs to be done, and is offering itself. Undoubtedly, our knowledge of the reflexes involved is very far from complete. In addition to local reflexes, there is the whole field of nervous control of the veins that has been relatively unexplored except for the classical work of Franklin and his colleagues. It is encouraging to see new interest and research in this direction.

Clinical application of the basic research might not be long delayed. If I may be permitted to present a series of one case—and I repeat, a series of one case—let me call attention again to Fig. 9, lower left hand corner. This was a case diagnosed as Buerger's disease, having had a complete unilateral sympathectomy, confirmed by the usual tests. As already mentioned, we studied the effects of posture on the toes of the operated side, to establish that the signs of the "venivasomotor" reflex were still present after sympathectomy.

As a matter of routine, we measured flows in the big toe of the unoperated side as control. The flow was very low indeed and there was no doubt that soon there would be trouble, perhaps calling for operation on that side. But we noticed from the records that although this toe was horizontal, in which case normally the veins are not full and there is no sign of this reflex, such signs were very marked in this leg. We have not published

this in the paper from which this slide is taken, but we then raised this unoperated leg to 15° and, to our astonishment, found that the blood flow increased many times; in fact, to values comparable with those in the sympathectomized limb. Undoubtedly, a vasoconstriction arising from the distension of the venous side of the circulation was playing an important role in producing the ischemia in this toe. Perhaps an exaggeration of the normal "venivasomotor" reflex, due to locally elevated venous pressure in narrowed venous channels, is important in such cases generally. We have had no opportunity to extend the measurements. Obviously, the effects of posture need to be measured quantitatively on patients with all types of peripheral vascular disease.

ARTERIOVENOUS ANASTOMOSES

I have to omit, for lack of time, discussion of the role of the arteriovenous anastomoses, which research is showing to be of the greatest importance Again, their physiologic control seems to be in the interests of the whole organism rather than of the local tissue. When the anastomoses open up, the total blood flow may greatly increase, yet the increase be non-nutritive, in that the oxygen supply to the tissues is not greatly improved. We have to raise the question whether some of our therapeutic procedures may not be increasing this shunt flow of blood without much benefit to the ischemic tissues

CONCLUSION

Throughout this lecture, I have tried to reiterate certain themes the peculiar subservience of the skin circulation to the needs of the whole organism, and, above all, the fact that so much more basic physiologic research is needed if we hope to understand, and so to manage, the manifestations of peripheral vascular disease. Treatment of the diseases themselves is a more distant goal. I am sure that Dr. George Brown, if he were here with us today, would emphasize how much more experimental research is needed on this fundamental physiology. There are enthusiastic and competent workers in the field, but their number is too few, and the necessary conditions for collaboration

between basic science investigators and their clinical colleagues, with access to patients for fundamental studies, are too often lacking. Perhaps it is to encourage such collaboration that the Chairman of your Section asked one who works at the basic level, far from the peripheral vascular clinic, to deliver this George Brown Lecture.

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2

*Factors that Determine the Rate of Venous Return to the Heart**

ARTHUR C. GUYTON, Jackson, Mississippi

The amount of blood pumped by the heart each minute is determined by two factors: first, the ability of the heart to pump blood forward and, second, the tendency for blood to return from the peripheral vessels to the heart. Because of the tremendous interest in cardiology throughout many years, the first of these factors, the ability of the heart to pump blood forward, has received much attention, and most of the diseases affecting the ability of the heart to pump blood are reasonably well understood. However, the second factor, the tendency for blood to return to the heart from the peripheral vessels, has received very little attention, and, as a result, little constructive information has yet been written relating to venous return of blood to the heart.

As long as the heart itself remains normal, it usually functions so efficiently that it immediately pumps all the blood which returns to it from the peripheral vessels. This is one of the general principles of the well-known Starling's law, and because of this effect the cardiac output normally is not determined to a

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major extent by the ability of the heart to pump blood but instead is determined almost entirely by the tendency for blood to return to the heart from the periphery. It is for this reason that the studies to be presented in this paper have been carried out. In other words, the purpose of these studies has been to elucidate the factors controlling venous return to the heart and thereby to elucidate the factors which control cardiac output.

THE "VENOUS RETURN CURVE" AND THE EFFECT OF RIGHT ATRIAL PRESSURE ON VENOUS RETURN

Figure 12 illustrates a "venous return curve" which depicts the effect of different right atrial pressures on the return of blood

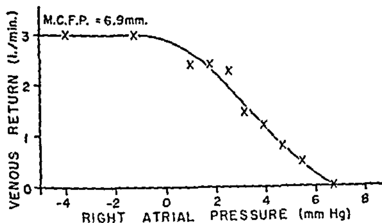


Fig. 12

to the heart. As long as the right atrial pressure is at least several mm Hg below zero, regardless of how much below zero it is, the return of blood from the peripheral vessels to the heart remains approximately constant.[2, 8, 7, 10] This causes the plateau that is evident to the left in each one of the venous return curves. The reason for this plateau is that when the right atrial pressure falls below zero, this tends to decrease the pressures in the veins as they enter the thoracic cage to values below 0 mm. Hg. How-

ever, the vessels collapse as the pressure in them falls to zero because of atmospheric pressure pressing against the overlying tissues. Therefore, the pressure in these collapsing vessels usually cannot fall below atmospheric pressure. Thus, regardless of whether the right atrial pressure is slightly below zero or 20 mm. Hg below zero, the return of blood to the heart remains approximately constant.

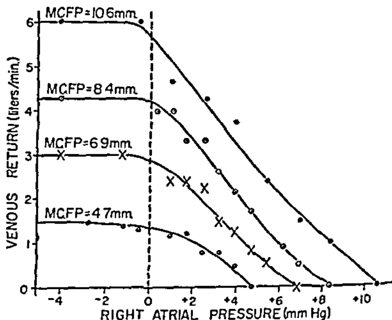


Fig. 13

When the right atrial pressure rises above 0 mm. Hg, the vessels entering the thoracic cage, instead of collapsing, become excessively filled with blood, and the rising right atrial pressure is transmitted backwards into the peripheral portions of the venous system. This backward transmission of pressure impedes the flow of blood from the peripheral vessels toward the heart and, therefore, decreases the venous return of blood to the heart. It is obvious from the venous return curve of Fig. 12 that the

greater the right atrial pressure, the less becomes the venous return, until finally a right atrial pressure is reached beyond which no blood at all returns to the heart.

EFFECT OF BLOOD VOLUME AND MEAN CIRCULATORY FILLING PRESSURE ON VENOUS RETURN

Four different venous return curves are shown in Fig. 13, the differences in these curves are due to differences in blood volume. These curves were all performed on the same dog one after another, the blood volume being increased between each two successive curves. It is evident that increasing the blood volume increases the venous return curves in all aspects. That is, at any given right atrial pressure, increasing the blood volume increases the venous return. The reason for this effect is that increasing the blood volume increases the distention of the blood vessels, and this in turn increases the pressures within each vessel. As a result, the pressure in the veins tending to force blood toward the heart is increased; the pressure gradient from the capillaries to the veins tending to force blood forward is increased, and, finally, the pressure gradient from the arteries to the capillaries tending to force blood forward is also increased. Thus, throughout the circulatory system the pressure gradients forcing blood toward the heart are increased as a result of elevated blood volume, and the resulting effect on the venous return curves is depicted graphically in Fig. 13.

"Mean Circulatory Filling Pressure" as the Upper Limit of Right Atrial Pressure

The *mean circulatory filling pressure* is the pressure in the circulatory system measured immediately after the heart has suddenly stopped pumping blood. To take accurate measurements of mean circulatory filling pressure, special procedures for bringing arterial and venous pressures to equilibrium within a few seconds after heart pumping ceases must be utilized Otherwise, because of many compensatory effects which occur after the heart stops pumping, the measured pressure is greatly different from the true mean circulatory filling pressure.[4, 8, 9]

Obviously, the mean circulatory filling pressure rises as the blood volume is increased, for this distends the circulatory system.

The mean circulatory filling pressure during the recording of each of the venous return curves of Fig. 13 is shown in the figure. This figure illustrates that the venous return of blood to the heart approaches zero as the right atrial pressure approaches the mean circulatory filling pressure. For instance, in the top curve of the figure the mean circulatory filling pressure is 10.6 mm. Hg, and venous return to the heart becomes zero when the right atrial

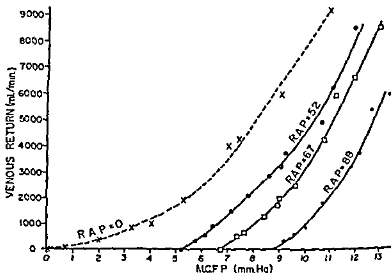


Fig. 14

pressure becomes equal to this value. Thus, the *mean circulatory filling pressure is the upper limit to right atrial pressure*[7] and the mean circulatory filling pressure in turn is determined by the volume of blood in the circulatory system and by the capacitance ($\frac{dV}{dP}$) of the vessels.[1,9] Therefore, the greater the blood volume in relation to size of the vascular bed and the distensibility of this vascular bed, the greater becomes the upper limit for right atrial pressure.

Figure 14 illustrates the effect of mean circulatory filling pressure on venous return when the right atrial pressure is maintained at a constant value. In each of these instances, it is evident that increasing the mean circulatory filling pressure increases the return of blood to the heart, and when the mean circulatory filling pressure falls to equal the right atrial pressure the venous return becomes zero.

"PRESSURE GRADIENT FOR VENOUS RETURN"

From the above principles of the effect of right atrial pressure and mean circulatory filling pressure on venous return of blood to the heart, a few general statements can be made regarding the effects of different pressures in the circulatory system on venous return: First, an increase in right atrial pressure opposes venous return provided the right atrial pressure is above 0 mm. Hg; second, an increase in mean circulatory filling pressure increases venous return; third, when the right atrial pressure approaches the mean circulatory filling pressure, the venous return approaches zero. From this it is evident that the return of blood from the periphery to the heart should vary approximately in proportion to the difference between the mean circulatory filling pressure and the right atrial pressure Additional research studies as well as a mathematical analysis of the circulatory system have shown this principle to be a valid one. Therefore, the difference between mean circulatory filling pressure and right atrial pressure is considered to be the *pressure gradient for venous return.*[2] However, any time the right atrial pressure falls below 0 mm. Hg, the right atrial pressure must be considered to be approximately 0 mm. Hg instead of less than this value because of the collapse of veins entering the thoracic cavity. Also, venous return is not always exactly proportional to mean circulatory filling pressure minus right atrial pressure because changing these two values can change the resistance to blood flow in the peripheral vessels, which will also affect venous return as will be discussed below. It is because of this effect that the curves of Fig. 14 are slightly curvilinear rather than absolutely linear as would be predicted otherwise.

EFFECT OF VASCULAR RESISTANCES ON THE RETURN OF BLOOD TO THE HEART

Figure 15 illustrates the effect on venous return of constricting the central veins transmitting blood into the heart.[3] It is evident from this figure that increasing the peripheral resistance of the circulatory system by this method greatly decreases the venous return of blood to the heart. In other words, increased venous

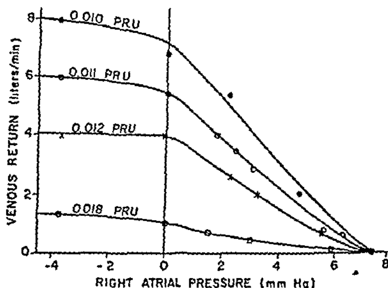


Fig. 15

resistance flattens the entire curve, decreasing the return of blood to the heart at each given value of right atrial pressure, whereas decreased venous resistance *increases* the return of blood to the heart for each given right atrial pressure.

It should be noted especially that all of the curves in Fig. 15 terminate at exactly the same point to the far right of the figure. The reason for this is that the mean circulatory filling pressure in each of these instances *remains constant*, and as discussed above it is the mean circulatory filling pressure that determines the upper limit to right atrial pressure.

Effect of Vascular Resistance in Different Portions of the Circulatory System on Venous Return

Referring once again to Fig. 15, one can see, by inspection of the different curves, that increasing the total peripheral resistance only 1 8 times as a result of contricting the central veins (bottom curve) decreased the venous return to a value 6 times below the

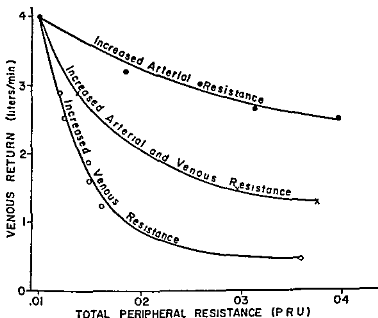


Fig 16

initial venous return when there was no constriction of the central veins (top curve) In other words, increasing the resistance in the major veins decreased venous return tremendously without affecting the total peripheral resistance to an exorbitant extent. Experiments have shown, on the other hand, that increasing the resistance in the arteries does not decrease the venous return to a major extent.[2, 5] This effect is illustrated in Fig. 16, which shows what happens to the maximum venous return when

the total peripheral resistance is increased by: (1) compressing the central veins; (2) progressively compressing medium-sized arteries and veins simultaneously; and (3) by plugging small arteries with minute glass beads. From this figure it is evident that increased peripheral resistance due to obstructed arteries decreased the venous return many times less than an equal increase in peripheral resistance caused by contricting the veins. Intermediate between these two was the effect of constricting both the veins and the arteries. These effects illustrate that changes in arterial and arteriolar resistance affect the total peripheral resistance greatly but do not greatly affect venous return. On the other hand, changes in resistance in the veins affect venous return of blood to the heart tremendously but do not affect the total peripheral resistance to a major extent. One of the most important implications of this finding is that cardiac output could be controlled mainly by vasomotor tone in the venous circuit while total peripheral resistance and arterial pressure could be controlled mainly by resistance in the small arteries and arterioles.

Effect of Epinephrine and Vasomotor Tone on Venous Return

Figure 17 illustrates the effects on the venous return curve of continuous epinephrine injection into a dog whose vasomotor reflexes had been totally abrogated by complete spinal anesthesia. This figure shows that epinephrine increases the venous return almost exactly the same as an increase in blood volume.[5] In other words, continuous epinephrine infusion increases the venous return of blood to the heart at each respective value of right atrial pressure. This effect almost certainly is due to increased mean circulatory filling pressure; it will be noted in Fig. 17 that the mean circulatory filling pressure rose from the control value of 11 mm. Hg up to 18 and 23 mm. Hg respectively during epinephrine injection at different rates.

Another very important effect is illustrated in the two top curves of Fig. 17—that is, at the higher rate of epinephrine injection the slope of the venous return curve is less than the slope of the venous return curve at the lower rate of epinephrine

injection. This effect would be expected for the following reasons: increasing the rate of epinephrine injection should increase vasomotor tone and therefore increase the mean circulatory filling presure as occurs in this figure, and, also, increasing the rate of epinephrine injection should increase the resistance to blood flow along the vessels which would decrease the slope of the venous return curve. Thus, epinephrine causes two effects on the venous return curve: one is due to increased mean cir-

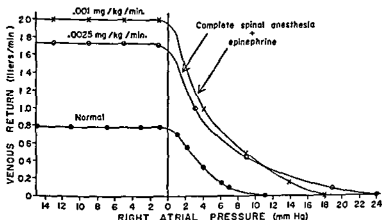


Fig. 17

culatory filling pressure, and the other is due to increased resistance to blood flow. From the study illustrated in Fig 17 it seems that lesser rates of epinephrine injection affect the venous return curve mainly by increasing the mean circulatory filling pressure, for the venous return curve at an epinephrine rate of 0.001 mg /Kg /min, is almost precisely the same as that recorded in Fig. 13 when the mean circulatory filling pressure is increased by increasing the blood volume. However, as the rate of epinephrine is increased still more, the resistance to venous return seems to increase progressively, and this opposes some of the beneficial effect on venous return afforded by the increasing mean circulatory filling pressure.

FORMULA FOR EXPRESSING THE EFFECT OF DIFFERENT FACTORS ON VENOUS RETURN

By mathematical analysis of the circulatory system[7] the following formula has been derived for expressing the effects of different factors on venous return:

$$VR = \frac{MCFP - RAP}{\dfrac{R_1C_1 + (R_1+R_2)\,C_2 + \ldots\ldots + (R_1+R_2+\ldots\ldots+R_n)\,C_n}{C_1+C_2+\ldots\ldots+C_n}}$$

in which *VR* is venous return, *MCFP* is mean circulatory filling pressure, *RAP* is right atrial pressure, *R* is the resistance of each respective portion of the circulatory system beginning at the central veins and proceeding backwards to the central arteries, and *C* is the capacitance $(\frac{dV}{dP})$ of each respective portion of the circulatory system beginning at the central veins and proceeding toward the central arteries. This formula illustrates that the venous return is proportional to the difference between mean circulatory filling pressure and right atrial pressure or, in other words, is proportional to the *Pressure gradient for venous return.* On the other hand, venous return is inversely proportional to a rather complicated factor shown in the denominator of the formula. This factor has been termed the *Impedance to venous return*, and the formula can be expressed in a simplified manner as follows:

$$VR = \frac{\text{Pressure gradient for venous return}}{\text{Impedance to venous return}}$$

From the denominator of the first formula, it can be seen that R_1 is far more important in determining the impedance to venous return than is R_n because R_1 continues to reappear over and over again in this expression of impedance to venous return. Because R_1 is the resistance in the central veins immediately adjacent to the heart, it is evident that resistances in the venous circuit closer to the heart are far more important in determining venous return than resistances progressively farther and farther away from the heart back toward the central arteries.

The value of each resistance in the denominator of the formula does not necessarily remain constant when some of the other values in the formula are changed. For instance, increasing the right atrial pressure increases the pressure in the central veins and distends these veins. Therefore, the resistances in the veins near the heart decrease while the resistances in all the other vessels might remain constant, rise, or decrease Also, changing the mean circulatory filling pressure changes the resistances throughout the circulatory system, for increasing the mean circulatory filling pressure increases the distention of essentially all vessels, and this in turn decreases the resistance. It is this effect of mean circulatory filling pressure on resistance that causes the curves of Fig. 14 to be curvilinear rather than linear; that is, when the mean circulatory filling pressure rises, not only does this increased pressure force increased quantities of blood back toward the heart because of *increased pressure gradient for venous return*, but it also increases the return of blood to the heart because of *decreased impedance to venous return* resulting from distended blood vessels.

The capacitance of each respective segment of the circulatory system is a measure of the ability of that portion of the circulatory system to store quantities of blood. When the capacitance is small and blood attempts to dam up in a particular vessel, the pressure in the vessel rises greatly. The increasing pressure then forces blood on through the resistances beyond the vessel and thus prevents excessive damming of blood in the vessel. However, when the capacitance is great large quantities of blood can be stored without causing excessive elevation of pressure.

The capacitance of the venous system is approximately 18 to 30 times as great as the capacitance of the arterial system.[1] For this reason the venous system can store much larger quantities of blood without exorbitant rise in pressure than can the arterial system. As a result, when resistances between the veins and the right heart impede the flow of blood from the veins to the heart, extreme quantities of blood are stored. But, when arteriolar resistance increases greatly and blood is dammed up in the arterial tree, the arterial pressure rises tremendously, this in-

creasing pressure to a great extent overcomes the resistance in the arterioles, allowing blood to flow from the arteries into the veins at a rate almost as great as usual. Therefore, it is very significant that the capacitance of the venous system is great and the capacitance of the arterial system is slight, for this effect is one of the major reasons why resistances in the venous circuit

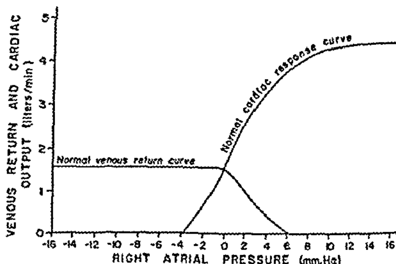


Fig. 18

affect venous return to a major extent while resistances in the arterioles and small arteries affect venous return to only a slight extent.

EQUATING VENOUS RETURN CURVES WITH CARDIAC RESPONSE CURVES

Except for a second or two at a time, the return of blood to the heart must equal the quantity of blood pumped out of the right atrium by the heart, or, in other words, the venous return must equal the cardiac output. This effect is illustrated in Fig. 18 which shows a normal venous return curve and a normal "cardiac response curve." The cardiac response curve is a form

of Starling's curve of the heart which depicts the ability of the heart to pump blood at different right atrial pressures Essentially all cardiac response curves have been obtained from isolated heart preparations, and the exact cardiac response curve of the intact dog or of the human being can only be inferred from many separate, isolated studies. The normal cardiac response curve as shown in this instance is approximately that obtained in this laboratory by injecting into the circulatory system of dogs large quantities of blood so that the right atrial pressure would increase.[5, 9] In some of these same animals the vasomotor reflexes had been abrogated by giving the animal total spinal anesthesia and then injecting epinephrine at a constant rate to take the place of the normal sympathetic impulses.

The approximate normal cardiac response curve as shown in Fig. 18 shows that cardiac output becomes essentially zero when the right atrial pressure is −4 mm. Hg because the introthoracic pressure is also about −4 mm. Hg. When the right atrial pressure becomes 0 mm. Hg, this pressure is approximately 4 mm. Hg greater than the introthoracic pressure, causing the heart to be filled with an "effective right atrial pressure" of approximately 4 mm. Hg. As a consequence of this, large quantities of blood empty into the right ventricle, and the heart then pumps the blood on into the arteries An average dog will pump about 1.5 liters of blood when the right atrial pressure is 0 mm Hg As the right atrial pressure increases above 0 mm. Hg, the cardiac output increases up to three or more times normal in the animal whose vasomotor and cardiac reflexes have been abrogated. If the reflexes are still intact, the curves are somewhat different as will be noted below.

We might consider for a moment the momentary effect of increasing the right atrial pressure to +4 mm. Hg in the system illustrated in Fig. 18. At this level of right atrial pressure, the venous return to the heart is 0 5 L./min., but the amount of blood pumped from the right atrium is 3.4 L /min. Thus, approximately 7 times as much blood is being pumped out of the right atrium as is returning to the right atrium. As a result, the total quantity of blood in the right atrium will be decreasing very

rapidly, and the right atrial pressure will be falling. As the right atrial pressure falls, the venous return rises to approach 1.5 L./min., and the amount of blood pumped out of the right atrium by the heart falls to approach 1.5 L./min. It is obvious, then, that should venous return and cardiac output only momentarily fall out of equilibrium with each other, within several beats of the heart this equilibrium will be re-established.

The point at which the venous return curve and the cardiac response curve equate determines (1) the cardiac output, and (2) the right atrial pressure both at the same time. Therefore, no single factor exists which determines cardiac output, and no single factor exists which determines right atrial pressure. Instead, both of these values are determined simultaneously by all the many factors that affect the venous return curve and all the many factors that affect the cardiac response curve. For this reason it is important to consider some of the conditions which can change the cardiac output and right atrial pressure.

Effect of Changing the Cardiac Response Curve on Cardiac Output and Right Atrial Pressure

Figure 19 illustrates two venous return curves and three cardiac response curves. The normal venous return curve and the normal cardiac response curve in an average dog equate at point *A* with a right atrial pressure of 0 mm. Hg and a cardiac output of 1.5 L./min. If the heart of this animal is damaged the cardiac response curve becomes that illustrated by the lower cardiac response curve. This depressed cardiac response curve equates with the normal venous return curve at point *B* so that the right atrial pressure becomes 2.7 mm. Hg and the cardiac output 800 ml./min. Thus, myocardial damage decreases cardiac output while at the same time increasing the right atrial pressure.

When the heart is stimulated strongly by the sympathetics it becomes considerably more efficient than normally,[6, 11] and the cardiac response curve becomes approximately that shown by the upper cardiac response curve of Fig. 19. This curve equates with the normal venous return curve at point *C*, at which point the right atrial pressure is −0.7 mm. Hg and the cardiac output

is 1600 ml /min. Thus, increasing the efficiency of the heart as a pump increases the cardiac output. However, this effect occurs only very slightly in the normal dog because the venous return curve reaches its plateau very rapidly after the right atrial pressure falls below zero, and once the venous return curve has

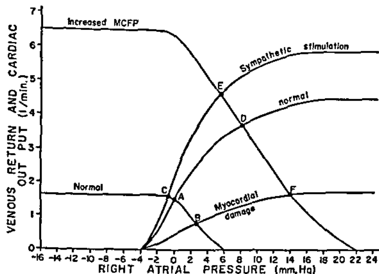


Fig. 19

reached its plateau, the heart can become infinitely efficient and yet the cardiac output still cannot increase any more.

Effect of Changing the Mean Circulatory Filling Pressure on Cardiac Output

Also illustrated in Fig. 19 is a venous return curve such as one obtains when the blood volume is greatly increased. The increased blood volume increases the mean circulatory filling pressure and therefore increases the venous return at all right atrial pressures. This increased venous return curve equates with the normal cardiac response curve at point *D*, illustrating that an excessive tendency for blood to return to the heart can cause

the right atrial pressure and cardiac output to rise. In this instance the right atrial pressure rises to 7.8 mm. Hg, and the cardiac output rises to 3.7 L./min.—2½ times normal—even though the operating efficiency of the heart itself has not changed. These are the effects observed in high cardiac output failure.

If the blood volume is greatly increased and the heart is simultaneously excessively stimulated by the sympathetics, then the venous return curve and the cardiac response curve will equate at point *E* so that the right atrial pressure becomes 5.2 mm. Hg and the cardiac output 4.6 L./min. It is evident then that increasing the efficiency of the heart as a pump increases the cardiac output considerably under these conditions and also decreases the right atrial pressure.

If the blood volume is greatly increased in a dog with myocardial damage, the two curves equate at point *F* with a right atrial pressure of 14.2 mm. Hg and a cardiac output of 1.6 L./min. In this instance the cardiac output is actually greater than normal because the increased blood volume has more than compensated for the depressed ability of the heart to pump blood. This illustrates very forcefully that the cardiac output is determined by the composite of factors affecting venous return and of factors affecting the ability of the heart to pump blood rather than factors affecting either one of these separately. Many patients with myocardial damage develop increased blood volume, and it is probable that this increased blood volume offsets some of the depression in cardiac output in exactly the same manner that increased blood volume can offset the effects of myocardial damage in a dog.

The mean circulatory filling pressure can also be increased by sympathetic stimulation or by epinephrine injection, and, as pointed out earlier in this paper, the increase in mean circulatory filling pressure resulting from these two factors is probably to a great extent comparable to increasing the blood volume, for the venous return curves obtained under such conditions are very similar to the venous return curves obtained in animals with increased blood volume. In patients with cardiac

disease extreme sympathetic activity is often noted. This increased sympathetic activity almost certainly helps to increase the return of blood to the heart and thereby overcomes some of the depressant effect of myocardial damage on cardiac output. However, the right atrial pressure is also greatly increased at the same time that the cardiac output is increased, just as occurs

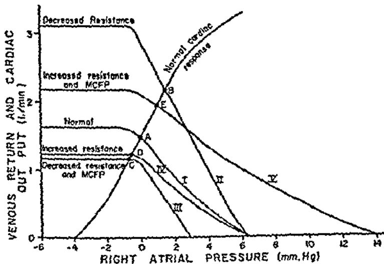


Fig. 20

when the blood volume is greatly increased in one who has a damaged heart.

Effect of Changing the Peripheral Resistance on the Cardiac Output and Right Atrial Pressure

Figure 20 illustrates a normal cardiac response curve, and it illustrates five venous return curves. The normal cardiac response curve and normal venous return curve equate at point *A*. If the mean circulatory filling pressure remains constant but the peripheral resistance is decreased, the venous return curve becomes Curve II which equates with the normal cardiac response curve at point *B*. At this point the cardiac out-

put has risen from the normal value of 1.5 L./min to 2.2 L./min, and the right atrial pressure has risen to 1.5 mm. Hg. Thus, decreased resistances in the peripheral vessels, especially in the peripheral veins, increases cardiac output and simultaneously increases right atrial pressure.

If the peripheral resistances are decreased as a result of decreased vasomotor tone, this ordinarily decreases not only the resistance to blood flow but also decreases the mean circulatory filling pressure for relaxed blood vessels can hold more blood than usual without developing elevated pressures. Therefore, depressed vasomotor tone is likely to cause a curve such as that shown by Curve III of Fig. 20. In this instance the cardiac output has fallen to 1.2 L./min., and the right atrial pressure has fallen to −0.7 mm. Hg. Thus, because factors that change the resistance to venous return usually also change the mean circulatory filling pressure, the net effects on cardiac output and right atrial pressure are determined by both of these factors acting simultaneously rather than by either one of the factors separately.

When the resistances in the peripheral vessels are increased, the venous return curve might become that illustrated by Curve IV which equates with the cardiac response curve at point *D*. In this instance the cardiac output is 1.25 L./min., and the right atrial pressure is −0.5 mm. Hg. Thus, increased resistances in the vessels decrease cardiac output and decrease right atrial pressure. However, the same factors which increase these resistances often at the same time increase the mean circulatory filling pressure. This is the effect that was illustrated in Fig. 17 following epinephrine injection, for epinephrine injection decreased the slope of the venous return curve—that is, increased the resistance to venous return—but at the same time increased the mean circulatory filling pressure. Curve V of Fig. 20 also illustrates the effect on venous return which one might expect to occur when the vasomotor tone throughout the systemic circulatory system is increased. This curve equates with the cardiac response curve at point *E*, causing a cardiac output of 1.9 L./min. and a right atrial pressure of 0.9 mm. Hg. Here again

the cardiac output and right atrial pressure are determined by *two changes occurring simultaneously in the systemic circulatory system* rather than by a single change.

SUMMARY

In summary, the relationship of venous return to cardiac output has been presented. The tendency for blood to return to the heart in each animal at any given instant can be expressed graphically by a "venous return curve," and the tendency for the heart to pump blood forward can be expressed by a "cardiac response curve." As illustrated by several examples in this paper, cardiac output and right atrial pressure of each animal at any given instant are determined by the point at which the venous return curve and the cardiac response curve equate.

The factors affecting venous return may be summarized as follows: (1) Right atrial pressures less than 0 mm. Hg have approximately the same effect on venous return as a right artial pressure of 0 mm. Hg because negative pressures in the right atrium cause collapse of the veins entering the thoracic cavity. (2) Increasing the right atrial pressure to positive values above 0 mm. Hg progressively decreases the venous return. (3) The upper limit of right atrial pressure is the mean circulatory filling pressure. (4) The mean circulatory filling pressure minus the momentary right atrial pressure is called the *pressure gradient for venous return*, and the venous return is proportional to this pressure gradient for venous return as long as the *impedance to venous return* remains constant. (5) Venous return is inversely affected by changing resistances in the peripheral blood vessels. Increasing the resistances in the arteries does not greatly decrease venous return, but increasing the resistances in the veins decreases venous return tremendously. (6) Increasing the blood volume or increasing vasomotor tone by epinephrine injection usually increases the venous return at any given right atrial pressure.

In a series of graphs the effects of different factors on the venous return curve and on the cardiac response curve are

shown. By equating these curves with each other, the effects of these factors on cardiac output and right atrial pressure are illustrated. Using such graphical procedures, and probably only by using these procedures or similar mathematical procedures, one can effectively analyze the control systems for cardiac output and right atrial pressure, both of which are simultaneously controlled by the equating of the momentary venous return curve and cardiac response curve of the animal.

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3

Physiological Aspects of the Regulation of Blood Volume

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My title may be somewhat misleading, as I do not intend to discuss the regulation of blood volume at all. The first aspect of any question to command my attention as a physiologist is the method of attack upon it This is the only aspect of my assigned topic that I shall try to discuss in the short time at my disposal.

Only a perfectionist would insist upon absolute standards of accuracy in the collection of physiologic data. For most purposes, we are more concerned with learning the direction and order of magnitude of changes than with establishing a precise static value. In the measurement of blood volume, I would not be greatly disturbed by an error of 10 or even 30 per cent if I had reason to believe that the error is constant I am, however, concerned with errors of any magnitude if the possibility exists that these errors may vary with physiologic conditions and thus mask, exaggerate, or imitate the changes we are trying to observe. I think it is important to keep alive the question, what exactly do we measure when we use modern methods for blood volume?

For the sake of brevity, I am going to make some categorical statements which I think will be accepted, on the basis of our present knowledge, without too much argument·

1. For practical purposes, the volume of the contents of the cardiovascular system as a whole can be measured only as the distribution space of injected materials. This, of course, requires the introduction of recognizable materials in known amounts, and measuring their concentration in a representative sample of blood after thorough mixing with all the blood has occurred.

2. The ratio of cells to plasma in a sample of blood withdrawn from the cardiovascular system does not, except by coincidence, represent the ratio in all the blood occupying the system.

3. No substance of limited diffusibility, and thus suitable for blood volume determination, exists in equal concentration in the cellular and the plasma compartments of blood. These two compartments must therefore be measured separately, the volume of cells as the distribution volume of tagged cells or cell solute, and the volume of plasma as the distribution volume of plasma solute.

The measurement of these two compartments is not equally easy. Whereas firm tagging of cells is theoretically possible, and seems to have been achieved with radioisotopes of iron and chromium, no comparable labeling of plasma as such is conceivable. An indefinitely long period may be allowed for firmly tagged cells to mix with the entire blood volume before a sample is drawn. It seems unlikely, at our present state of knowledge, that any recognizable substance that could be introduced into the plasma compartment would both mix uniformly throughout the compartment and remain confined to it in similar fashion. The actual rate of loss of plasma solute from the circulation as a whole cannot be measured until thorough mixing throughout the system has taken place. Assumptions therefore have to be made about the rate of loss during the early period following injection.

In a review on blood volume written in 1921, Joseph Erlanger said, "At what rate the substance will disappear during (this) period, it is impossible to say." Despite the accumulated data of a third of a century, culminating in recent elegant mathematical analyses of the problem of simultaneous mixing and disappear-

ance, what was impossible in 1921 seems equally impossible in 1954.

Some years ago, my colleagues and I raised the question whether the rapid decline in concentration of the circulating dye T-1824 for the first several minutes following injection is entirely due to mixing within the circulation. If most of the injected dye still remains within the circulation at the end of this

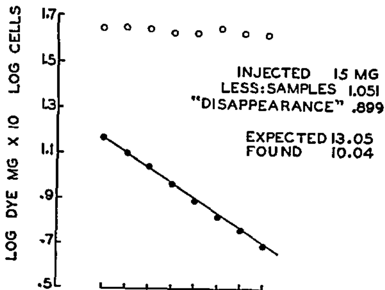


Fig 21

time, it seemed to us that it should be possible to recover it by a suitable washout procedure.

Figure 21 shows a typical washout experiment, in which dye was washed out by periodic bleeding and replacement with undyed whole blood. The sloping line connects the successive yields of the dye, T-1824. Since the yields form an exponential series, it is possible to calculate the total amount of dye which would be obtained if the series were continued indefinitely. The balance sheet for this experiment is shown at the upper right. In

studies of this kind, corrected for dye removed in the sampling and observed to disappear with time after the rapid disappearance phase is over, only about 80 per cent of the expected dye was recovered. The open circles above in the figure show the rather constant yield of cells obtained when dye is washed out with whole blood.

Now, it is obvious that in these experiments, we are washing out only those portions of the circulation whose contents can mix with the injected blood in the interval between replacement and the next bleeding. In the experiment shown, this interval was three minutes. Incomplete recovery of dye might mean only that some dye is in sluggish circuits, does not mix with the injected blood, and is therefore not washed out. This possibility was tested in other experiments by making the intervals alternately three minutes and ten minutes, as shown in the next figure.

In Fig. 22 the length of each interval is indicated at the bottom in minutes. Had the shorter intervals been insufficient for mixing, larger dye yields would have been expected at the end of the ten-minute intervals, and the data for the ten-minute series would lie above the three-minute slope. This is obviously not the case. All the data lie on a single line. In this particular instance, recovery of dye was 66 per cent.

Since these slopes indicate that about 15 per cent of the available dye is being bled out each time and the circulating dye is depleted so rapidly, a correction for disappearance of the remaining dye with time does not significantly change either the slope or the total yield. The only correction of this sort that can be used is the disappearance rate observed just before the washout is started. Of course, this may not be valid, since the massive bleedings and infusions in the washout procedure—10 cc. Kg. for each bleeding and replacement—may so disturb the circulation as drastically to change the rate of dye disappearance. In controlled studies on this question, however, we have obtained no evidence that this is the case. If the blue dye, T-1824, is injected into a dog and allowed to pass through the initial phase of rapid disappearance and into the slower,

exponential phase of disappearance, withdrawal and re-injection of these volumes of blood does not alter the disappearance slope.

In an extension of these studies, we compared the behavior, in the circulation, of dyed and undyed plasma injected in equal volumes and under identical conditions. Dyed plasma was prepared either by adding dye to plasma *in vitro*, or by injecting a donor dog with massive doses of T-1824. In order

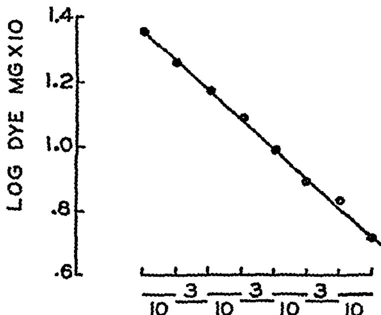


Fig. 22

to observe the behavior of undyed plasma, it was, of course, necessary to make it distinguishable from the plasma already in the circulation. To accomplish this, we colored the circulating plasma with an injection of dye into the dog an hour or more in advance. The addition of undyed plasma to the circulation accordingly produced a lowering of the circulating dye concentration.

Figure 23 compares the time: concentration curves obtained following these two types of plasma injection, the one dyed, the other undyed. From the two curves on the left, in the same dog, it is apparent that the large volumes of predyed plasma, shown in the upper curve, are handled by the circulation in much the same way as the usual small volume of aqueous dye solution, shown below. The early phase of rapid disappearance lasts approximately 15 minutes in this dog, for both dye and dyed plasma. The data on the right show that undyed plasma

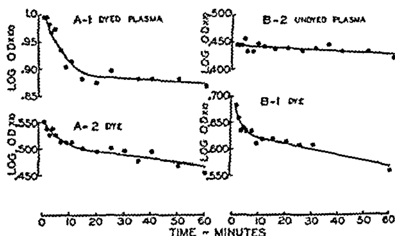


Fig. 23

behaves quite differently. In this animal, whereas the phase of rapid disappearance following dye injection, shown at the bottom, lasted approximately 10 minutes, no such phase is recognizable from sampling at one-minute intervals following the injection of undyed plasma. If we are to attribute the excessive early fall in dye concentration to mixing, we should have to conclude that undyed plasma mixes, in this instance, within one minute.

Figure 24 shows another injection of undyed plasma into a predyed dog at time 0. In this case, what might represent a mixing phase is recognizable as a temporary excessive fall in

plasma dye concentration below the later levels. In no case have we seen such a phase lasting longer than three minutes.

If allowance is made for the volume of injection, it is possible to calculate the circulating plasma volume from the injection of dye, from the injection of dyed plasma, or from the injection of undyed plasma into a predyed dog. The procedures and the calculations with the injection of dyed and of undyed plasma are identical, except for algebraic sign, the former giving an increase and the latter a decrease in dye concentration.

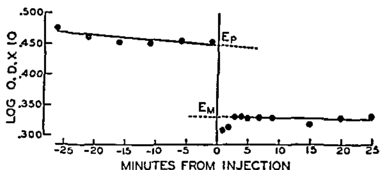


Fig. 24

Figure 24 shows an extrapolation through the very brief mixing period to a concentration E_M This extrapolation is identical in its assumptions with the procedure used in the dye method for obtaining a mixed dye concentration at the instant of injection. Whereas plasma volume calculated from the injection of dyed plasma is in good agreement with that obtained with conventional dye injections, the volume calculated from undyed plasma injection is always smaller, by an average of some 20 per cent. Furthermore, the latter smaller value, obtained with undyed plasma injection, is in fairly good agreement with the distribution volume of dye as calculated at one minute following injection, that is to say, long before the initial phase of rapid disappearance is completed.

Although these are old data, and I have nothing new to add,

I think the conclusions to be drawn from them are important enough to justify their review here. The conclusion seems inescapable that under the conditions of our experiments, dyed and undyed plasma behave quite differently in the circulation. In the light of our knowledge of the binding of T-1824 by plasma albumin, this must mean either that this linkage is broken within the circulation, or that the circulation does not treat dyed and undyed albumin in the same way. By any interpretation, the apparent distribution volume of the dye in these experiments is greater than that of undyed albumin.

Having played the devil's advocate in attacking the dye method for measuring plasma volume, let me add that I know of nothing better. I am impressed with data showing that the distribution volume of T-1824 is the same as that of other macromolecular plasma solutes having quite different properties. It is conceivable that anomalous dye distribution in our studies is attributable to our choice of experimental conditions. It seems just as likely to me, however, that only under optimum conditions does the dye or any other plasma solute actually measure the plasma compartment. A completely unbiased observer might wonder whether the size of this compartment can be measured under any conditions.

4

Use of Different Dye Methods in Blood Volume Determination

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In 1915 Keith, Geraghty and Rowntree introduced the dye method for determining plasma volume using Brilliant Vital Red. Since that time more than 600 scientific papers on this subject have been published throughout the world. Nevertheless there are still a number of unsolved problems, some of which will be discussed here.

1. The chemical substances originally used, Brilliant Vital Red and Congo Red, are rather rapidly eliminated from the blood stream. At the present the most commonly used dyes are T-1824 (Evans Blue) and Trypan Red both of which offer satisfactory results These two dyes are largely bound to the serum albumin. My associates Zissler and Schneider have determined the plasma volume of 24 healthy male medical students Using Trypan Red an average plasma volume of 2893 cc, or 43 0 ± 4.0 cc./Kg. body weight was obtained and with T-1824 a plasma volume of 2854 cc. or 42 6 ± 3 5 cc./Kg. body weight. Two simultaneous tests were made on the same individual, first injecting the Trypan Red and 1 hour later the T-1824 or vice versa and it has been found that the plasma volume figures are identical in both instances.

Usually the dyes are evenly mixed within less than three minutes, as shown by blood analyses from arteries and veins and from the abdominal vessels in test animals. Somewhat longer mixing times are rarely encountered, and only in certain pathologic conditions. Similar mixing times have been determined for iodine^{131}-tagged albumen and for radioactive red cells. Between the third and eighth minute after dye injection the analysis usually shows stationary values, while after the eighth minute the dye concentration gradually decreases.

2. The optimal period for sampling the dye analyses represents a very important problem which is still under investigation and which makes it difficult to compare the results of different research workers. We determine the plasma volume from several blood samples taken between the third and sixth minute following the dye injection and we require identical values in at least two samples. Other workers have selected longer intervals up to 30 minutes or more, and have tried to obtain the assumed basic value 0 at moment of injection by using the linear extrapolation method. It is my belief that this method is inadequate for the following reasons:

A. It is impossible in these longer periods to determine the amounts of dye that are excreted by the liver or in the lymph stream of the thoracic duct or that enter the cells of the reticuloendothelial system.

B. From the eighth to tenth minute after injection the rate of decline of dye concentration is variable in different individuals—in some steep, in others rather gradual in a shallow curve—and in others the concentration remains at the same level up to the thirtieth minute (Fig. 25). At the present we do not know the clinical significance of these three types of curves and cannot correlate them with different cardiovascular conditions. However, these curves show that the linear extrapolation method will lead to extremely variable and therefore erroneous results because the selected points on the curve are chosen at random.

C. The last and most important factor in favor of the short time method is not based upon technical reasons but upon a specific physiologic consideration. Joseph Barcroft has shown

by the *carbon monoxide method that blood volume in dog and* man does not have a constant and invariable ratio to body weight, but is subject to continual short-interval fluctuations. Barcroft has developed concepts concerning the blood depots and reserves in the body which, however, have not been generally accepted. It is certainly true that these so-called blood reserves that Barcroft reports in the spleen are not completely separated from the general circulation. In our first paper con-

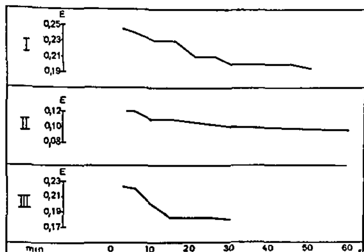


Fig 25

cerning this subject in 1927 we demonstrated the reserve-function of the subpapillary skin capillaries and the constant interchange between the rapid blood flow of the general circulation and the sluggish stream in the dilated capillary network. Barcroft, Benatt, Greeson, and Nisimaru confirmed our observations concerning the subpapillary skin plexus and showed that after the tenth minute tagged blood slowly enters the dilated capillaries. Therefore I have suggested that one should speak of *the active blood volume* and of *the blood reserve,* factors that are determined or defined and influenced by the

great local variations in the velocity of the blood. Within the first 8 minutes the injected dyes are mixed predominantly in the active blood and after this time the amount of dye disappearing into the dilated reserve depots become appreciable. It is therefore obvious that in using the long time factors one will obtain in addition to the active blood also blood originating from the reserve depots.

Recently we have again attempted to investigate this problem experimentally (Fig. 26). In 8 test subjects in recumbent position we determined the plasma volume by means of the dye T-1824 and checked the plasma dye concentration during the following 30 minutes at frequent intervals. Following this, tourniquets with subsystolic pressure were applied to both thighs and the subjects positioned by means of a tilt table in a 45° Fowler position. The dye concentration in the venous blood remained almost unchanged. A second determination of the plasma volume in the same position after repeated dye injections, however, showed a decrease of the plasma volume of about 400–1000 cc.

In a second series of 6 healthy students in supine position the plasma volume was determined before and 30 minutes after intravenous injection of 0.25 mg. k-strophanthin. Here too the decrease of the plasma volume of about 200–500 cc. was not recognized immediately from dye concentration curves but only following a second dye injection.

In a third series of 11 healthy individuals the plasma volume was determined in a sitting position with tourniquets applied to both thighs. The dye concentration in the plasma was determined in regular short intervals and after 30 minutes, with the tourniquets removed and the tilt-table brought into horizontal position, the dye concentration in the plasma decreased, indicating an increase of plasma volume by 200–600 cc. The repeated determinations following a second dye injection revealed the same quantitative increase of the plasma volume. Brown, Hopper, Sampson, and Mudrick showed with carbon monoxide a similar decrease of the blood volume after 5 minutes. The blood volume changes, however, were considerably less if the

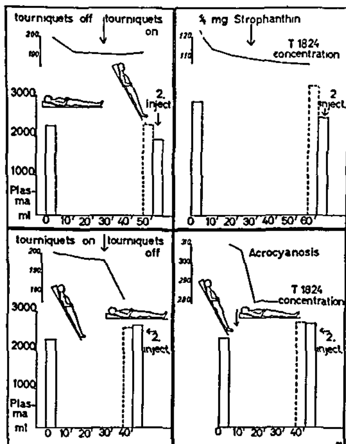


Fig. 26

analyses were made after 20 minutes. The calculated blood volumes after this time were greater. The results of these experiments indicate that both dye and CO mix within the first 6 minutes with the active blood and thereafter slowly enter the depot created by the tourniquet in detectable amounts.

In patients suffering from functional capillary disturbances with acrocyanosis and with manifestations of the orthostatic

syndrome, the same findings are observed. Three patients suffering from this syndrome were put on the tilt-table and remained for 15 minutes in the high Fowler position at a 75° angle, after which the plasma volume was determined and the patients placed in horizontal position. Five to ten minutes later the plasma dye excretion curve revealed a decrease in the concentration. The plasma volume increases because of the influx of undyed plasma from the predilated capillaries. The second test-analysis following repeated dye injections shows an increase of 300–400 cc. in plasma volume after 30 minutes. Displacement of the blood from the lower extremities can be achieved not only by change of position but also by bandaging the legs in a cephalad direction.

These experiments clearly demonstrate that a decrease in the plasma volume can be recognized only after a second dye injection and escapes recognition if the concentration curve only is considered. The increase of the plasma volume, however, can be recognized from decline of the concentration curve as an indication that undyed plasma from the reserve depots has entered the blood stream. But in this case, too, a second analysis after repeated dye injections proves to be much more reliable. All functional changes are demonstrable only by the use of short time samples. It is my feeling that these experiments fully prove the existence of the active blood volume and in turn furnish indirect proof of the presence of a reserve blood depot within the capillary system of the extremities.

3. Another problem is the calculation of the blood volume by means of the hematocrit. Somewhat smaller blood volumes are produced by using radioactive red cells as the determining factor than by the dye method and the hematocrit. From this it can be concluded that the venous hematocrit values seem to be too high and that the so-called body-hematocrit gives lower values. The red cell content in the capillaries is supposedly lower than in the arterial or venous blood stream. It was therefore proposed to introduce a correction factor of 0.87–0.92. Moreover, 20 years ago, the carbon monoxide method (which is used in red-cell determinations in the same way that radioactive isotopes

are used) resulted in consistently lower blood-volume values than the dye methods. Steinmann, as well as Hopper, Tabor and Winkler, later improved the carbon monoxide method and reported agreement between this and dye method. According to our experiments hematocrit values in man from the capillary, venous, and arterial blood and in the dog from the vena cava and the brachial artery are identical (Fig. 27). Deviations from these identical values are found only in hematocrit readings

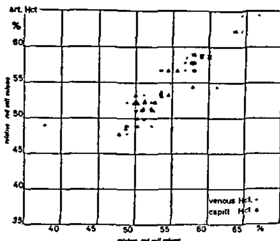


Fig. 27

below 25 per cent and over 70 per cent and in dogs in profound shock preceding death. In these cases of shock, however, the internal organs exhibit increased hematocrit values. I am therefore inclined to believe that this correction factor is of somewhat doubtful value and that the so-called body hematocrit is not fully acceptable at the present stage of our investigation. This whole problem, however, appears to be rather unimportant from a clinical point of view because the correction factor will change the values in both normal and pathologic conditions in the same direction.

A survey of normal values obtained by the various methods

during the past 25 years shows rather small and insignificant variations from each other (Fig. 28). The calculated plasma volumes lies between 2654 and 3350 cc. Our own observations—comprising two research periods ten years apart—on 300 healthy individuals have shown average values of 2720 cc. and 2702 cc. respectively, or 42 cc. and 41 cc. per kilogram of body weight.

Authors	Method	n	Plasma vol. ml	Plasma vol. ml/kg	Red cell vol. ml	Red cell vol. ml/kg	Blood vol. ml	Blood vol. ml/kg
Rowntree Brown a Roth	Vital red 3-6'	74	3350	51			5710	87
Seyderhelm a Lampe	Trypan red 3-6'	9		44				83
"	Trypan blue 3-6'	11		45				82
Wollheim (1927-1939)	Trypan red 3-6'	194♂	2720	42			5400	83
Gibson a Evans	T 1824 3-35'	49♂	2948	43			5335	78
Zissler a Schneider	T 1824 3-4-6'	107	2702	41	(2383)	(36)	5085	77
Storaasly a a	J^{131}-Albumin 10'	31♂	3001	40			5391	71
Kaplan a a	J^{131}-Albumin 10-30'			43		30		73
Steinmann	CO 5-9'	8					5412	81
Hopper, Tabor a Winkler	CO 15-20'	9				35	4998	80
"	T 1824 10'	9		46			5037	81
Hevesy a a	P^{32} Ery 5-10'	21			2107	33		
Nylin a Hedlund	P^{32} Ery >60'	19			1945	29		
Reeve a Veall	P^{32} Ery T 1824 20(-30)	13	(3359)		2162		5521	
Gibson a a	Fe^{59} Ery T 1824 10-20'	40	(3552)	48	2209	30	5761	77
Sterling a Gray	Cr^{51} Ery 10-20-30'	25♂			2351	32		
Gray a Frank	Cr^{51} Ery 10-20-30' Cr^{51} Plasma	10	2795	41	2081	30	4876	71

Fig. 28

The whole blood volume figures show somewhat greater variations. In general it has been found that long analysis times revealed higher values than short analysis times.

4. I want to mention briefly a few observations and results in clinical cases as regards the dye method in plasma volume determination. During the past years we had been especially interested in the changes in the blood-volume in anemias and leukemias. For example, a plasma volume of 2918 cc. and a red-cell volume of 495 cc. was found in a case of macrocytic anemia in a 52-year-old white female in the first dye analysis before treatment. Following administration of vitamin B_{12} a steep rise of the reticulocyte count up to 59 per cent took place.

On the same day the dye analysis and the hematocrit reading revealed a red-cell volume of 789 cc., *an increase of 60 per cent* compared to the original pretreatment values (Fig. 29). This increase coincides exactly with the rise of the reticulocytes.

Moreover, repeated plasma determinations are of particular value and importance in massive intestinal hemorrhages, of which *we have encountered 35 during a period of a few years.*

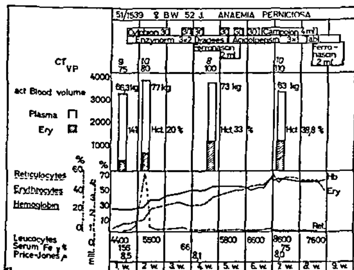


Fig. 29

The red-cell figures and haemoglobin values are often misleading in the initial stages of shock Only determination of the blood volume will serve as a true indicator of the severity of the blood loss, its cessation or persistance, and the effect of therapeutic measures.

In 1928 and 1931, we reported in detail on blood volumes in the different types of cardiovascular decompensation. To date we have collected 560 such patients, 401 of which exhibit what we call a *plus-decompensation* (Figs. 30 and 31). This type of decompensation is characterized by an increased active blood

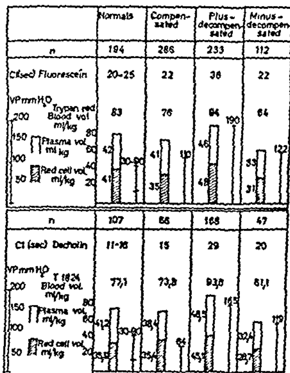


Fig. 30

		Plasma volume ml	Plasma volume ml/kg	Blood volume ml	Blood volume ml/kg
Normals	87 ♂	2746	40,9	5215	77,6
"	20 ♀	2529	43,0	4439	75,3
average	107	2702	41,2	5085	77,1
Plusdecompensation	115 ♂	2918	48,2	5805	95,4
"	53 ♀	2569	48,7	4797	89,1
average	168	2805	48,5	5460	93,8
Minusdecompensation	18 ♂	2480	33,0	4831	83,2
"	29 ♀	2259	32,1	4029	80,0
average	47	2343	32,4	4332	81,1

Fig. 31

volume, high venous pressure, dyspnea in the horizontal position and improvement by sitting with the legs dependent, restlessness, and insomnia I want to emphasize that the active blood volume expresses the volume of the venous blood returning to the heart. The determining factor in the occurrence of decompensation is the disturbance in the relation between the active blood volume and the minute output of the heart. It is obvious that in the case of decompensation the end result will be the same whether a sudden drop in the minute output occurs or the active blood-volume rises without change in the minute output. Simple consideration of this mechanism may perhaps simplify the controversy between the advocates of forward-failure and backward-failure.

In some cases we have observed that the rise in the active blood volume precedes the manifest clinical signs and symptoms such as visceral congestion, dyspnea, and dependent edema by several days. It is therefore possible to produce experimental heart failure by increasing the active blood volume artificially, provided that the heart is not able to increase its output correspondingly.

I feel that the immediate and primary reason for the increased active blood volume is anoxemia or CO_2 accumulation in the peripheral circulation. I want to point out that sodium retention, which has come into the foreground in recent years, plays only a part in the later and fully developed stages of heart insufficiency. The clinical picture of "plus-decompensation"—increase of active blood volume and true heart insufficiency—will show decrease of the blood volume as soon as improvement sets in (Fig. 32). On the other hand we recognize a second type of decompensation, which we have called *minus-decompensation*, in which a primary decrease of active blood volume exists. Both types of decompensation are encountered in the same primary cardiovascular conditions, such as organic valvular lesions, hypertension, and cor pulmonale. Between 20 and 30 per cent of all decompensations fall into this second group of minus-decompensation, in which the venous pressure is low or normal and the veins poorly filled. The patients are most com-

fortable in the horizontal position and do not benefit from sitting upright in a chair, as dyspnea and other symptoms will increase, in contradistinction to patients suffering from plus-*decompensation. As soon as the decompensation subsides the active blood volume will rise.* This "minus-decompensation" is usually found in acute myocardial infarctions and it is of par-

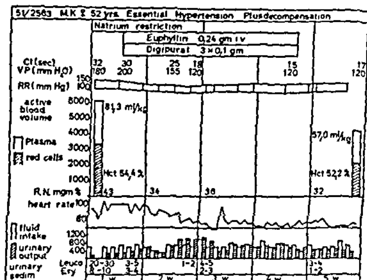


Fig 32

ticular importance in these cases to recognize the presence or absence of initial shock by determining the active blood volume (Fig. 33).

Some workers in the U. S. A. and Europe have found essen-*tially the same data which we have discussed:* Goldbloom and Libin, Gibson and Evans; Meneely and Kaltreider; Nylin and Hedlund; Prentice *et al.;* Berlin *et al.;* Schreiber *et al;* Reilly *et al.;* Kaplan *et al.*

We may call all *circulatory conditions in which the venous return to the heart and the active blood volume are decreased, diminished "peripheral circulatory insufficiency."* The colori-

metric blood volume determination enables us to distinguish three types of this insufficiency: simple hypovolemia, shock syndrome with hemoconcentration, and vasomotor collapse in which there is profound drop of the arterial pressure indicating involvement of the vasomotor regulatory mechanism Of course it is understood that these types of peripheral circulatory insufficiency may also occur in the non-diseased heart.

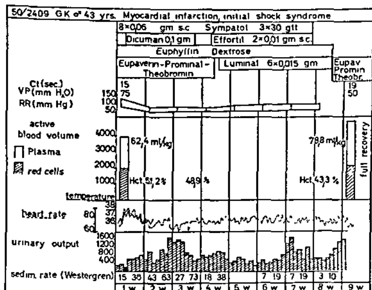


Fig 33

Finally it must be pointed out that this dye test has proven that in shock the replacement of the active blood volume by plasma transfusion is more effective than an equal amount of whole blood. It has also been shown that strophanthin and all digitalis preparations with the exception of lanatoside C will cause an *acute and immediate decrease* of the active circulating blood volume and that on the other hand the sympathomimetic drugs will cause hypervolemia. This has greatly facilitated the specific use and selection of the various cardiac drugs.

I believe that I have been able to demonstrate the usefulness of this dye-plasma test, which can be readily performed as a routine laboratory procedure, which may be repeated in short intervals, and which is sufficiently accurate to give valuable information as to the underlying mechanism and the prognosis of a heart condition.

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5

Comparison between Different Radioactive Labeling Technics for Measuring Red Cell Volume: The Use of Radioactive Chromium

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My assignment this morning is to review the radioactive methods of measuring red cell mass, with particular emphasis on our own studies with radioactive chromium.

The radioactive labeling technic of measuring red cell volume is based upon calculating the dilution of a known volume of radioactive red blood cells that have been injected into the subject or experimental animal and allowed to mix thoroughly in the circulation—"the isotope dilution principle."

Ideally, a technic that offers the opportunity of measuring red cell mass should fulfill a number of different criteria: The tagging should be permanent. There should be no leakage from the red cell into the plasma, and no retagging of cells from the plasma. The method must be accurate, simple, and rapid. The half-life of the isotope should be practical, depending somewhat upon the problem at hand.

The method should permit the transfusion of the subject's own tagged cells, thus avoiding incompatability complications, blood

storage problems, and the possibilities of serum hepatitis, all of which are inherent in tagging a donor's blood. The radioactive technic, of course, must be safe from the chemical and radioactive points of view, and should allow repeated injections and determinations.

Doctor Nylin has studied a number of isotopes from this point of view: phosphorus, sodium, potassium, zinc, iron, glycine tagged with nitrogen, carbon, and recently thorium B, a gas that is converted to radioactive lead upon entering the red cell.

Shortly after the second World War the Biophysics Laboratory at Harvard, under Doctor Baird Hastings, wanted someone to work in the field of radioactivity, who was primarily a clinician, with some experience in physiology and biochemistry. When I was given the appointment I knew so little about biophysics, or radioactivity, that I thought the easiest approach would be to start with a new isotope which had never been used before. Just by good fortune, it turned out to be radioactive chromium

Radioactive chromium, Cr^{51}, is a soft x-ray emitter with a half-life of 26.5 days It distintegrates by K capture with transmutation to vanadium and emission of x-rays of 4 92 Kev plus approximately 10 per cent of 0.237 Mev gamma rays.

Two forms of radioactive chromium were prepared. the anionic hexavalent radioactive sodium chromate ($Na_2Cr^{51}0_4$) and cationic trivalent radioactive chromic chloride ($Cr^{51}Cl_3$). The radioactive sodium chromate tags the red cells, and the radioactive chromic chloride labels the plasma proteins (Figs. 34, 35) Radioactive sodium chromate tagged red cells and radioactive chromic chloride may be injected simultaneously into the blood. The two chemical forms of the same isotope selectively render the red cells and plasma proteins radioactive without significant cross tagging, permitting the accurate measurement of the red cell mass and plasma volume simultaneously with an error of about 3 percent, as verified by hemorrhage and transfusion experiments in man and animals

The red cells contain about 20 γ per cent of chromium and the plasma 14 γ per cent. There are trace amounts of chromium in all the organs of the body as determined by chemical analysis.

When radioactive sodium chromate is added to erythrocytes in vitro, it is taken up avidly by the red cells, which retain their radioactivity without significant loss to the plasma for periods of one day or more after injection into experimental animals or

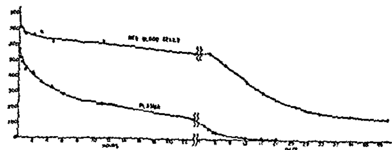


Fig. 34. Distribution of Anionic Cr^{51} ($Na_2Cr^{51}O_4$) in the Red Blood Cells and Plasma of the Dog.

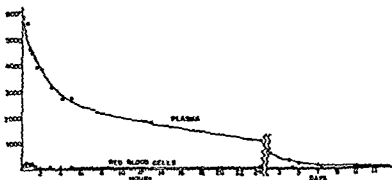


Fig. 35. Distribution of Cationic Cr^{51} ($Cr^{51}Cl_3$) in the Red Blood Cells and Plasma of the Dog.

humans. This marked affinity of the erythrocytes for the anionic chromate ion is demonstrated by the rapid uptake of radioactive chromium following the addition of $Na_2Cr^{51}O_4$ to saline suspensions of washed red cells (Fig. 36). The prolonged retention of radioactivity by the red cells without significant loss to the saline medium is presented in Fig. 36. In a series of such experiments 60 to 90 per cent of the isotope was found to be bound to the red cells within two hours.

Washed red cells, tagged with sodium chromate and injected into a human or experimental animal, retain their radioactivity without significant loss to the plasma for periods of 24 hours or more. Since the exchange of Cr^{51} between the red cells and plasma is negligible for 24 hours or longer, radioactive chromate has proven to be ideal for the tagging of red blood and the measurement of circulating red cell volume.

A small aliquot of the subject's erythrocytes are tagged in

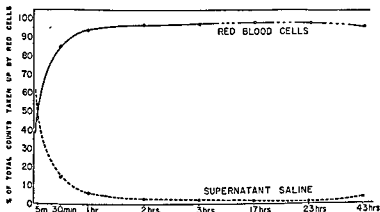


Fig. 36. Uptake of Anionic Cr^{51} ($Na_2Cr^{51}O_4$) by Human Red Blood Cells.

vitro with radioactive sodium chromate and then reinjected. After the tagged cells have mixed in the circulation, the radioactivity of the subject's erythrocytes may be determined and the red cell volume calculated by the isotope dilution principal. The red cells are then hemolyzed and counted in the liquid phase in a scintillation counter. The low radioactivity dosage (total dose of 0.1 rep or less) permit repeated use of the Cr^{51} method in the same individual.

The red cell volume in ml. may be calculated using the quotient:

$$\frac{\text{Total counts in tagged erythrocytes injected}}{\text{Counts per ml. packed red cells in the subject's circulation}}$$

The radioactivity of the circulating red blood cells remains constant for approximately 24 hours without appreciable loss to the plasma. Repeated determinations of the red cell volume are reproducible within 5 per cent for approximately 24 hours after the injection of Cr^{51} tagged erythrocytes. The accuracy of the Cr^{51} method as verified by hemorrhage and transfusion experiments with measured volumes of blood is within 3-5 per cent.

The Cr^{51} method of determining circulating red cell volume combines the accuracy of the radioiron technic with the simplicity of the radiophosphorus method. The preparation of red cells tagged with radioiron necessitates the administration of radioiron for one or more weeks to volunteer donors who incorporate it into the hemoglobin of their red cells, which must be then transfused into the experimental subject. This may be contrasted to the instantaneous tagging of the subject's own red blood cells with sodium chromate. The red cell mass determined by the Cr^{51} method was 31.8 cc./Kg. body weight and 1.21 L./sq.m. surface area compared to 29.7 cc./Kg. and 1.15 L./sq.m. as measured with radioactive iron (Table 1).

The red cells mass values determined by different isotope technics are presented in Table 2. The chromium method yields slightly higher values than radioiron, but lower than radioactive

TABLE 1. Comparison of Normal Circulating Red Cell Volume Determined by Radioactive Chromium and Radioactive Iron

	Total (cc.)	*cc./Kg. body weight*	*L./sq m. surface area*
Cr^{51} determinations (25 students)	2351 (±290)	31.8 (±3.5)	1.21 (±0.12)
Radioactive iron determinations (40 students)	2208	29.7	1.15

TABLE 2. RED CELL MASS VALUES WITH DIFFERENT ISOTOPE TECHNICS

ml.	*ml./Kg.*	*Isotope*
2351±290	31.8±3 5	$Na_2Cr^{51}0_4$
2081±508	30.3±5.6	Combined chromium method
2208	29.7	Fe^{59}
2550	33.7	P^{32}
2353	34 5	CO
2380		Thorium B

phosphorus and carbon monoxide. The red cell mass in milliliters measured by $Na_2Cr^{51}0_4$ was 2351 (±290), almost identical with the thorium B results, 2380 (Table 2).

The chromium method shares with the P^{32} technic the advantage that a small sample of the subject's own blood may be tagged rapidly in vitro and then reinjected. In contrast to P^{32}, however, the Cr^{51} tagged cells retain their activity without significant loss to the plasma for approximately 24 hours after injection. The loss of radioactivity from the red blood cells in one hour (8 per cent) with radiophosphorus may be compared with a loss of 1 per cent in 24 hours with radiochromium (Table 3). With thorium B, there is no loss in 2 hours, but an appreciable loss occurs within 24 hours, according to Nylin.

The accuracy of the Cr^{51} method, as verified by hemorrhage and transfusion experiments with measured volumes of blood,

TABLE 3. RADIOACTIVITY LOSS FROM LABELED RED BLOOD CELLS AFTER INTRAVENOUS INJECTION

Labeling isotope	*Loss* (%)	*Time* (hr.)
P^{32}	8	1
Thorium B	0	2
$Na_2Cr^{51}0_4$	1	24

was within 3–5 per cent, comparing favorably with other isotope methods. If an unknown amount of blood has been lost after the initial red cell volume determination, a second injection of tagged erythrocytes is necessary. The red cell volume may then be calculated by dividing the total counts in the second injection by the difference in counts per milliliter of packed red cells between the samples obtained before and after the second injection.

The Cr^{51} method of measuring red cell mass was used extensively in evaluating hemorrhage and shock during the Korean War. The low radioactivity dosage (total dose of 0.1 rep or less) permits repeated use of the Cr^{51} method in the same individual. The amount of chromium injected is negligible.

FRACTIONATION OF RED BLOOD CELLS PREVIOUSLY TAGGED WITH RADIOACTIVE SODIUM CHROMATE

To ascertain what component of the erythrocyte contained the radioactivity, red blood cells previously tagged with $Na_2Cr^{51}O_4$ were fractionated into stroma-free hemoglobin, globin HCl, hemin, and washed stroma, and the radioactivity of the intact red cells and the red cell fractions was compared. The counts were calculated on the basis of 1 ml. of packed red cells (Table 4).

The activity of the hemoglobin (stroma-free) was 97 per cent of that of the red cells, indicating that hemoglobin was the

TABLE 4. FRACTIONATION OF TAGGED RED BLOOD CELLS

Blood fraction	*Packed red cells* (counts/cc.)	*Percentage distribution*
Red blood cells	19,700	100
Hemoglobin		
(stroma-free)	19,150	97
Globin HCl	15,400	78
Hemin	0	0
Washed stroma	398	2

important factor in red cell binding (Table 4). The recovery of 78 per cent of the activity in the globin fraction and only a trace in hemin suggests binding of the chromium by the globin portion of hemoglobin. The presence of only 2 per cent of the radioactivity in the washed stroma confirms the importance of hemoglobin in red cell uptake.

When stroma-free hemoglobin was prepared from red cells previously tagged with $Na_2Cr^{51}O_4$ and then dialyzed against saline for three days, 63.5 per cent of the radioactivity remained with the hemoglobin within the bag. The tagged hemoglobin, moreover, retained its activity after 24 hours of mixing with a combination of anionic and cationic exchange resins capable of removing all of the chromium present.

The site of tagging then appears to be within the hemoglobin of the erythrocyte (97 per cent), particularly in the globin fraction. Only 2 per cent of the counts were recovered in the washed stroma (Table 4).

A study of the kinetics of the uptake of sodium chromate by red cells indicated that the anionic sodium chromate diffused through the cell membrane and was then bound firmly by the hemoglobin, probably after reduction to the cationic trivalent state within the red cells. The intact red cells do not take up a significant amount of cationic trivalent chromium ($Cr^{51}Cl_3$) in vivo or in vitro. The failure of $Cr^{51}Cl_3$ to tag the intact erythrocyte is attributed to the relative impermeability of the red cell membrane to cations.

USE OF RADIOACTIVE SODIUM CHROMATE IN DETERMINING SURVIVAL OF HUMAN ERYTHROCYTES

The tagging of red blood cells by sodium chromate is so firm that the loss per day after reinjection approximates 1.0 per cent (Table 5). This firm binding has made it possible to measure red cell survival by a simple radioactive technic using $Na_2Cr^{51}O_4$, which compares favorably with the more complicated agglutination method of Ashby. The mean erythrocyte decay rate for both methods is identical −0.91 per cent per day (Table 5). The survival time of transfused heterotype blood, and the life

TABLE 5. Comparison of Erythrocyte Decay Rates as Measured Simultaneously by the Ashby and Cr^{51} Methods

	Ashby Method		Cr^{51} Method	
Case No.	*Loss* (%/da.)	*Length of Study* (da.)	*Loss* (%/da.)	*Length of Study* (da.)
1	0.88	97	1.0	85
2	0.82	113	0.85	105
3	0.91	103	0.85	112
4	0.98	99	0.91	107
5	0.95	91	0.91	100
Average	0.91		0.91	

span of the patient's own red blood cells determined by selective agglutination and Cr^{51} counting yield practically identical results. (Figs. 37, 38).

The advantages of the $Na_2Cr^{51}O_4$ method over the agglutination technic in respect to simplicity, accuracy, and time are obvious.

The opportunity of determining the survival time of the patient's own blood by reinjecting his own tagged red cells has

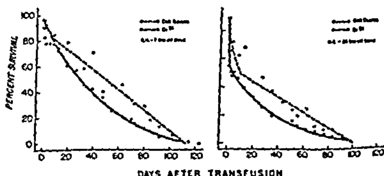


Fig. 37. Comparison of Red Cell Survival Data Obtained by Selective Agglutination and Cr^{51} Counting.

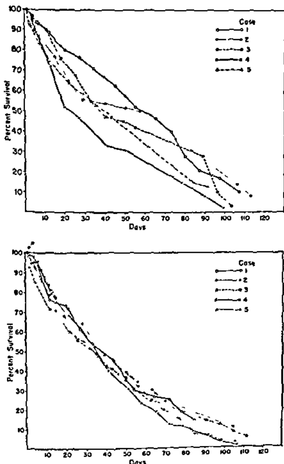


Fig. 38. Erythrocyte Survival of Transfused Heterotype Blood by Radioactive Chromium Technic.

made possible extensive studies in hemolytic anemias, liver disease, high altitude problems, shock, circulation problems, heart failure, survival of transfused and stored blood, and many hematologic problems.

SIMULTANEOUS DETERMINATION OF RED CELL MASS AND PLASMA VOLUME WITH RADIOACTIVE SODIUM CHROMATE AND CHROMIC CHLORIDE

The anionic form of chromium, $Na_2Cr^{51}O_4$, labels the red blood cells, while the cationic hexavalent chromic chloride is firmly bound by the plasma proteins both in vivo and in vitro. When chromic chloride is injected intravenously, 98 per cent or more is bound immediately by the plasma proteins with minimal loss of the isotope to the red cells. Once the chromic chloride is bound to the plasma proteins, it can leave the circulation only at the slow rate at which proteins leave it (Fig. 39). The dilution of this tracer substance in the circulation after intravenous administration was used to measure the plasma volume in man. The circulating plasma volume may be calculated by the isotope dilution principle, correcting for the loss of protein-bound radioactive chromic chloride from the circulation by a curve extrapolating the radioactivity to zero time (Fig. 39).

The red cell mass and plasma volume may be measured simultaneously by tagging a small aliquot of the subject's erythrocytes with radioactive sodium chromate and reinjecting the labeled cells. Ten minutes later a sample of blood for the red cell mass determination is obtained, and a saline solution of radioactive chromic chloride is then injected into the circulation. Four samples of blood are then taken at 5-minute intervals from the opposite arm, and the counts per milliliter of plasma are plotted on semilogarithmic paper. A straight line is obtained, and the theoretical counts per milliliter of plasma at zero time can be extrapolated. (Fig. 39). The plasma volume is calculated by the formula:

$$\text{Plasma volume} = \frac{\text{Total counts injected} - 2 \text{ per cent}}{\text{Counts/ml. plasma at zero time}}$$

The 2 per cent subtracted from the total counts represents the average maximum counts lost to the erythrocytes, reflecting the specific affinity of chromic chloride for the plasma proteins. The

bond of sodium chromate to the erythrocytes is sufficiently strong to prevent loss of radioactivity to the plasma.

A second plasma volume, such as following hemorrhage or transfusion, requires a second chromic chloride injection. A significant number of counts may still be present in the plasma at the time of the second injection of chromic chloride. In the

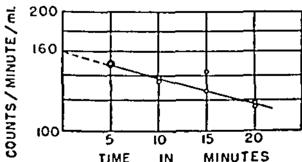


Fig. 39. Dilution Curve of Radioactive Chromic Chloride in the Circulation.

calculation of the final plasma volume these retained counts are subtracted from the counts in the plasma after extrapolation to zero time to obtain the corrected counts.

$$\text{Second plasma volume} = \frac{\text{Total counts in 2nd injection} - 2 \text{ per cent}}{\text{Corrected counts/ml plasma at zero time}}$$

The plasma volume determination using radioactive chromic chloride presents a simple, accurate, and rapid technic, by which a stable, nontoxic chemical solution can be injected directly, obviating the necessity of tagging albumin or other proteins in vitro. Determinations can safely be performed repeatedly and in rapid succession. Self-absorption corrections are not necessary. Liquid phase gamma ray counting permits the measurement of the plasma volume within a few hours

From a practical standpoint, Cr^{51} has several advantages. Lipemia and hemolysis do not interfere with the determination.

In vitro tagging of proteins is unecessary, since 98 per cent or more of an injected dose of chromic chloride is immediately bound by the plasma proteins in vivo. The simple chemical solution of chromic chloride is stable for long periods. The low dose of radioactivity (0.1 rep.) permits repeated use of the Cr^{51} method on the same individual, and the amount of chromium (0.1–1.0 mg.) used in a single experiment is also sufficiently low to permit repeated administration with complete safety. Results of the determination are available within six to seven hours if a Geiger or proportional flow counter are used, for which the planchets must be dried. When the samples are counted wet in a gamma ray counter, the final results are available within one to two hours. This makes the technic a rapid and useful clinical tool. Furthermore, this method makes possible the simultaneous determination of plasma volume and red cell mass with two forms of the same isotope, radioactive chromic chloride and sodium chromate. The two forms of the isotope may be present in the circulation simultaneously without interfering with the accuracy of either the red cell or plasma volume determination.

The accuracy of the method is within 3 per cent, as verified by hemorrhage and transfusion experiments with measured volumes of plasma and red cells. The mean values of the red cell mass and plasma volume obtained by the combined method agree favorably with those obtained when radioactive sodium chromate and chromic chloride are administered separately (Table 6).

TABLE 6. PLASMA VOLUME DATA WITH DIFFERENT ISOTOPE TECHNICS

Isotope	*ml.*	*ml./Kg.*
$Cr^{51}Cl_3$	2894±360	39.3 ±4.9
Combined chromium method	2795±498	41.1 ±5.6
T-1824	2948	43.08±5.9
Iodinated albumin	3002	40.6 ±4.4

The mean plasma volume value of 39.3 ml./Kg obtained by the Cr^{51} method agrees closely with the iodinated albumin technic—40.6—and with the T-1824 method—43.1—within one standard deviation (Table 6).

The ratio of red cell mass/plasma volume obtained by the combined Cr^{51} technic (0.91) closely approximated that determined by the use of the Ashby and T-1824 methods (0 89), and

TABLE 7. Ratio of Red Cell Mass to Plasma Volume as Determined by Different Isotope Technics

Isotopes	*Red cell mass / Plasma volume*
CO / T-1824	.93
Fe^{59} / T-1824	.87
P^{32} / T-1824	.87
Ashby / T-1824	.89
$Na_2Cr^{51}O_4$ / $Cr^{51}Cl_3$	0 91

was comparable to the values of 0 87 with the use of radioactive iron or phosphorus and T-1824 (Table 7). The body hematocrit:

$$\frac{\text{Red cell mass}}{\text{Red cell mass} + \text{plasma volume}}$$

averaged 42 3, and the ratio of the body hematocrit to the venous hematocrit was 0 91 (± 0.10), identical with that obtained by radioiron and T-1824 (Table 8).

TABLE 8. Ratio of Body Hematocrit to Venous Hematocrit as Determined by Radioactive Iron and T-1824 and by Radioactive Sodium Chromate and Chromic Chloride

Isotopes	$\frac{\textit{Body Hct}}{\textit{Venous Hct}}$
$\frac{Fe^{59}}{\text{T-1824}}$	0.91
$\frac{Na_2Cr^{51}O_4}{Cr^{51}Cl_3}$	0.91

In summary, then, the radioactive isotopes offer great potential in the study of red cell mass, and the newer technics now make it possible to evaluate red cell mass and plasma volume simultaneously with good accuracy.

6

Physiologic and Clinical Applications of the Isotope Technic in Blood Volume Determinations

P. WASER, Basel, Switzerland

The labeling of red blood cells or plasma with radioactive isotopes has been a great help to physiology and clinical diagnosis. Some of the reasons have been made clear in the previous papers. However, let us keep in mind the accuracy obtainable with a certain method Physiology demands a very exact technic, usually time consuming, while approximate values may be sufficient for clinical diagnosis. For the former purposes, all technics of blood volume determination that use the clinical hematocrit are inexact. This also applies to the technic of measuring the activity of whole blood samples after injection of labeled erythrocytes or even whole blood The reason for this inaccuracy lies in the difference between venous and total body hematocrit, which results in different dilution of the tracers in both the red cell and the plasma compartment. The most exact way to determine the whole blood volume is to measure the volume of red blood cells with marked erythrocytes, then measure the volume of the differentially labeled plasma, and add results This is shown in Dr. Gray's paper.

The technics for labeling red cells or plasma referred to in the preceding papers have proved their reliability in many comparative measurements.

CHOICE OF TRACER

Three criteria must be satisfied in our choice of a radioactive tracer: first, the effective half-life of the tracer in the body. That means the radiation dose actually given by the isotope to the body. Second, the minimal radiation intensity indispensable for the measurements; and, last, the hemodynamic half-life, which we define as the time necessary for the tracer in erythrocytes or plasma to be reduced by half, independent of the mode at diminution, exponential or otherwise.

There are isotopes produced for tagging the red cells; and for tagging the plasma. Phosphorus diminishes to half its value within one day; potassium takes a little longer to leak out; chromium is a very stable and very good tag; but the best one is, naturally, iron, incorporated in the heme part of the hemoglobin; and, according to my calculations from the data of Dr. Nylin, thorium B must have a hemodynamic half-life of around two and a half days.

For plasma, I^{131} has usually been selected and, also, of course, the chromium discussed by Dr. Gray. I am glad to tell you that another method is now available. We can use I^{132}. This has big advantages. This isotope has a much better graduation, it is several gamma quanta stronger than I^{131}, and it is easily available out of Te^{132}, which you can get at the pile; in fact, tellurium decomposes with a half-life of 77 hours to I^{132}. You can easily distill the I^{132} out of the iodate solution after reducing it to iodine and then, adding albumin, run the whole thing through a sterile ion exchange column; two hours later you have a marvelously labelled albumin for the plasma determination.

Naturally, a short physical half-life is also to be desired if the experiments are to be repeated. This is especially well satisfied by I^{132}, because it has a half-life of only two and a half hours. Measurements can be repeated within half hours or hours.

The mixing rate of the erythrocytes is relatively short, about

one to five minutes, for plasma, much longer—thirty minutes—presumably due to the marginal film of the fluid in the vessels and the slow stagnating flow in the capillaries. These facts should be kept in mind in blood volume determination with tracers.

PHYSIOLOGIC RESULTS OBTAINED WITH THE TRACER TECHNICS

A very important biologic factor studied by Nylin and coworkers is the volume of erythrocytes in the normal adult, which is approximately 31 Gm./Kg. in a number of investigations. This matches beautifully with other determinations. In opposition to earlier authors, who believed that it would increase, it has been found that no change greater than around 4 per cent takes place in the volume of circulating erythrocytes even during bodily exercise or after injection of epinephrine. Hahn and coworkers have shown this with Fe^{59}, Nylin and coworkers with P^{32} labeled erythrocytes, while Hunzinger and Bigne recently found in a preliminary study with I^{131} albumin that the plasma volume is not increased after injection of adrenergic Veritol These experiments show that the whole blood volume of man is not altered by physiological stress or epinephrine. Moreover, the human body, unlike the dog, does not have any significant blood depots

The circulating blood volume of an organ can be accurately determined by measuring the dilution of the tracer before and after temporary exclusion of the organ from circulation. Using this method on a few cases, Nylin reported 14 per cent of the blood volume to be in the legs and 16.7 per cent in the left lung.

CLINICAL APPLICATIONS OF BLOOD VOLUME DETERMINATIONS

The following clinical problems of blood volume have been thoroughly investigated with the help of radioisotopes.

The red cell volume is increased in *polycythemia vera.* The difference between absolute and relative polycythemia can easily be shown. Here especially, the usual clinical hematocrit

gives much too low results, so that tagging forms the only accurate method.

Hedlund found an increase of total red cell volume in congestive heart failure, in cases with increased residual blood in the ventricles. This is most pronounced in cases of congenital heart defects, such as the tetralogy of Fallot, with 8000 Gm. red cells, or patent ductus arteriosus. This fell to normal levels postoperatively.

On the other hand, red cell volume is decreased *in anemia, pernicious anemia,* and *tuberculosis.*

Important contributions have been made to the explanation of the mechanism of *shock.* Using Fe^{59} tagged erythrocytes Hahn found in hemorrhagic, oligemic shock, an instantaneous compensation for the lost blood by an increase of plasma to the normal whole blood volume. Later, plasma volume decreased proportionally to the increase in regenerated cells. The red cell hematocrit increased, the isotope concentration in the red cells diminished by dilution, because of the formation of new red cells, but the isotope concentration in the whole blood, naturally, remained constant. Transfused erythrocytes are not deposited in the spleen or the bone marrow in significant amounts.

In *normovolemic shock,* about a fifth of the labeled cells stagnate in the capillary system, as shown by Fine, Seligman and Gibson.

Dilution curves of normal subjects with P^{32} tagged erythrocytes show that mixing takes place within 20 to 30 seconds, and is constant after one minute. Since in normovolemic shock *neither the erythrocytes nor the whole blood volume* are decreased, and Nylin showed that the mixing time for the P^{32} labeled red cells is much longer—75 minutes—than the normal 1 to 5 minutes, it is clear that the blood stream must be slowed down in the capillary system. Large amounts of blood are pooled in spleen, liver, lungs, muscle, and mesenteric vessels.

The effective circulating blood volume is diminished, but only this portion of the total blood volume is affected. An additional loss of plasma through the capillary walls into the tissues could not be shown by Fine and Seligman with the help of S^{35},

Br^{82}, and I^{131} labeled plasma proteins. Only in the traumatized region are the capillaries more permeable. This loss of plasma volume cannot explain shock, except in the case of large burns or some other types of trauma

Hunzinger and Willenegger in Basel investigated compensation of blood loss with plasma substitute. Plasma and blood volume were determined a week before the transfusion with I^{131} tagged albumin, for exact measurement of the albumin clearance. This was tested again shortly before the experiment. Then, an infusion of sodium chloride, of plasma, and of the plasma substitutes, was made, and blood volume was determined repeatedly about 6 hours later. The result demonstrated clearly that only transfused plasma remains for a long period of time in the vascular system. Sodium chloride or subsidone infusions remained only for a very short time, 15 minutes Other plasma substitutes, such as peristone or dextrane, remained for about 4 hours. All these plasma substitutes differ in macromolecular distribution. The red cell volume is not changed.

In the same way it can be shown that passage through the vessel walls by these plasma substitutes or by sodium chloride is not decreased by rutin or adrenoxyl.

There has been until today only one good plasma substitute, and that is more plasma. We need a plasma substitute with a molecular weight between 50,000 and 100,000 The weight cannot be too large, because then the osmotic pressure will not be big enough, and it cannot be too low because then it would be excreted too easily.

7

Residual Volume of Blood in the Right and Left Ventricle

ROBERT F. RUSHMER, Seattle, Washington

The basic concepts of cardiac function and control have been derived from controlled experiments on anesthetized, thoracotomized animals. One principle of cardiac control is expressed by Starling's law of the heart which states than an increased energy release by the heart is attained by increased diastolic distention of the chambers. Although this law has been confirmed repeatedly under specific experimental conditions, the importance of its role in intact animals and man has been questioned.

In 1923, Meek and Eyster[13] computed the volume of the heart from roentgenograms exposed near the end of diastole and near the end of systole and concluded that the output of the heart could be increased by either an increase in diastolic volume or by a greater systolic ejection. Although these observations were contrary to the responses observed in heart-lung preparations, little interest was aroused. We are indebted to Nylin and his associates for a quantitative analysis of the relation between the heart volume and the stroke volume in normal human subjects with various types of heart disease.[14] They established values of normal heart volume,[11] and studied the changes occurring as a result of various conditions including muscular exertion.[19] They

advanced the concept that a given stroke volume can be obtained with less diastolic distention than occurs at rest. This suggested the possibility of hormonal effects capable of modifying Starling's law during muscular exercise. A detailed analysis of indicator dilution curves as a function of time permitted calculation of blood in the heart and lungs. Some 29–30 per cent of the total blood volume appeared to be contained within these compartments of the circulation. The importance of the heart as a blood reservoir has also been stressed by other investigators employing roentgenographic technics.[7,9,20]

Bing[1] estimated the diastolic and systolic volumes in the right ventricle, using a double lumen catheter in the right ventricle and pulmonary artery. Evans blue dye was injected into the right ventricle and dye dilution curves were recorded from samples withdrawn from the pulmonary artery. The accuracy of the method was assessed using two different artificial circulations. The computed values for right ventricular volume at the end of systole varied from 23 to 160 cc. The average value was 50 cc./sq. m. body surfaces, which was consistent with the values obtained by Nylin[14] and by Friedman.[2] Average values indicated that the amount of blood remaining in the right ventricle at the end of systole exceeded the quantity ejected (residual volume/stroke volume = 1.75).

Clearly these studies of cardiac function and control on man disclosed responses which are inconsistent with predictions based on observations on isolated or exposed hearts of animals, as embodied in Starling's law of the heart. A recent symposium indicated the extent to which the applicability of Starling's law to "normal" conditions is now being questioned.[3,6,8,12,15,17,19] For such reasons, it seemed imperative to obtain direct measurements of the important parameters of cardiac function in intact unanesthetized experimental animals during spontaneous activity.

METHODS

Continuous measurements of left ventricular diameter have been recorded by means of variable inductance gauges mounted across the chamber as indicated in Fig. 40B. The technic of in-



Fig 40A



Fig 40B

stalling this type of gauge has been previously described In another series of animals, left ventricular circumference was indicated by variable resistance gauges consisting of delicate rubber tubes filled with mercury and sealed at the ends with wires When the rubber tube is stretched, the electrical resist-

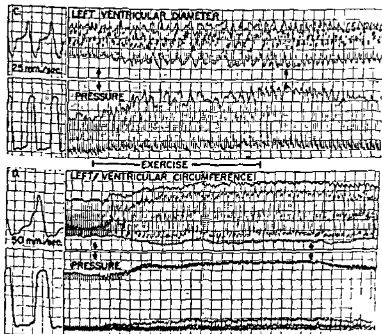


Fig 40C and D

ance of the mercury column is heightened because its cross sectional area is reduced and its length is increased

During installation, one of the lead wires was passed into the right ventricular cavity so that it followed the contour of the interventricular septum (see Fig 41) When the two wires were then fastened together the gauge encircled the left ventricular cavity and registered ventricular circumference by changes in length. Effective left ventricular pressure was recorded by a

small differential transformer pressure gauge installed at the apex of the heart with a short polyethylene catheter passing into the left ventricular cavity. The other end of the gauge was exposed to intrapleural pressure After recovery from the operation, records were obtained at frequent intervals as long as the gauges continued to function (over intervals as long as 26 days).

RESULTS

Changes in left ventricular dimensions and pressures have been recorded during a wide variety of spontaneous activity, including eating, sleeping, startle responses, and exertion Considerable variability has been encountered in the responses of different animals to similar conditions and in the same animal on different occasions. Space does not permit a detailed account of these observations However, the responses of two different dogs to a similar degree of exertion will serve to illustrate some points which are germane to this symposium. In one animal (Fig. 40C), the response to exercise (trotting 90 yards) was characterized by more complete systolic ejection as indicated by a smaller systolic diameter The slight change in diastolic diameter was accompanied by greatly increased diastolic ventricular pressure. Another animal, performing the same exercise, consistently displayed a predominate increase in diastolic circumference with a slightly increased systolic ejection. In these responses, an increased stroke volume was apparently attained by increased diastolic distention, greater systolic ejection, or a combination of the two. Occasionally, repeated exercise experiments in the same dog on the same day demonstrated predominant diastolic distention on one record and predominant systolic ejection on the next. The same type of variability has occurred in response to the injection of neurohormones (epinephrine and acetylcholine) All of our measurements have reaffirmed the concept that the ventricles normally function at large diastolic and systolic dimensions such that considerable quantities of blood remain within the cavities at the end of systole

Clearly, augmentation of stroke volume by more complete systolic emptying involves ejection of a greater amount of the so-called residual blood. Since the quantity of blood remaining

in the ventricular chambers at the end of systole is varied under different conditions, it serves as a reserve volume which can be utilized to increase stroke volume. For this reason, a new nomenclature has been derived for ventricular volumes which indicates their functional significance.[16] The extent to which a ventricle can be distended beyond the resting level constitutes

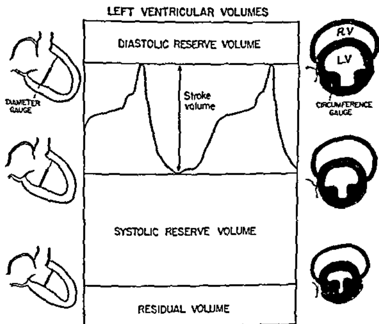


Fig 41

the *diastolic reserve capacity* (Fig. 41). The degree of diastolic filling is determined by the effective filling pressure and the resistance to stretch of the ventricular walls (ventricular distensibility). The *systolic reserve* represents the additional volume of blood which can be ejected by maximal ventricular emptying. The degree of systolic emptying is determined by the systolic ventricular pressure and the myocardial contractility, defined as the amount of myocardial shortening per unit of intraventricular pressure. The small quantity of blood remaining in the ventricles

at the end of maximal ventricular ejection is labeled *residual* volume. The terminology indicated in Fig. 41 is similar to that used to describe the lung volumes and has the same logical basis.

SUMMARY

The discovery that large quantities of blood remain within the ventricular cavities at the end of systole in both man and experimental animals, has prompted a re-evaluation of the mechanisms for increased cardiac output. The traditional concepts are based on the notion that the ventricles are small at rest and increase in both systole and disastolic dimensions to eject a large stroke volume. In contrast, measurements in intact animals and man indicate that the ventricles function at large dimensions at rest and increase stroke volume by greater diastolic distention, more complete systolic ejection, or both. The cardiac response is so variable under different conditions that multiple mechanisms must be involved. It is postulated that at least five variables are involved in establishing stroke volume: (a) heart rate, (b) effective filling pressure, (c) resistance to stretch of ventricular walls (distensibility), (d) degree of myocardial shortening (contractility), and (e) arterial blood pressure. Evidence is available indicating that each of these variables can be influenced by autonomic control or by circulating neurohormones (epinephrine or acetylcholine).

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PART II

Contractile Protein in Heart Muscle

8

Introduction

MAURICE B. VISSCHER, Minneapolis, Minnesota

This symposium is on "The Physiology of the Contractile Protein in Heart Muscle." We chose this subject for a symposium because of the growing importance of newer knowledge of the contractile proteins in muscle, including heart muscle, and because of a conviction that information about these newer developments in muscle and heart muscle physiology is of importance to the thinking of every cardiologist, particularly every investigative cardiologist.

The intimate mechanism by which chemical potential energy is translated into mechanical energy by the heart has been and still is somewhat obscure, as you will realize after listening to the morning program. It is, however, one of the crucial problems in pure and applied cardiac physiology, because if we are to have any rational basis for approaching the problem of increasing the energy output by the heart or of increasing the fraction of liberated energy that can be put to useful work, it will help us a great deal to have information about the character of this linkage between the chemical potential and the mechanical energy that does the work that the heart has to do.

We have all realized since it was first demonstrated that the heart did work in moving blood around the vascular bed, that there would have to be found a mechanism for translating chem-

ical energy to mechanical work. Over the intervening centuries —actually, since William Harvey and Stephen Hales—a large number of theories as to how chemical energy goes to mechanical work have been proposed, tested, and found wanting.

The reason for our difficulty, I think, has been, largely, the inadequacy of methods of study of protein structure, enzyme reaction kinetics, and the substrate end-product analysis in the microsystems with which we have a deal.

The long-term background of our knowledge of the contractile proteins is something like this: It is about a century since Kühne discovered that large quantities of a viscous protein, which he named at that time "myosin," could be extracted from muscle by strong salt solution. But more than half a century elapsed before any really significant developments in connection with the characteristics of myosin began to be seen, and they depended upon the development of new physical and physicochemical tools for the study of myosin. For example, optical studies of birefringence told us something about the shape of the molecule or molecules in what was extracted as myosin, but no one had anything more than the vaguest intuition that myosin, because of the large quantity found in muscle, must have some peculiar function in connection with contraction until the studies of Engelhardt and his collaborators in the late 1930s demonstrated that myosin had an ATP-splitting activity. This link, then, with the high-energy phosphate-bond metabolism was a major factor in stimulating the new work that has gone on since then.

The Engelhardt group also made some observations with regard to the effect of adenosine triphosphate, ATP, on the physical characteristics of myosin, but it remained for Szent-Györgyi and his associates to develop work along this line in a more extensive way.

I want to mention also another chapter or two in the history of physiologic investigation in this field which has a bearing on our subject although not concerned primarily with contractile proteins. I refer to the "three revolutions in muscle physiology."

Those of you who have read the physiologic literature of the last twenty years know that one of the most productive workers

in the field of muscle physiology, A. V. Hill, referred to the changes that occurred in our thinking about muscle physiology in the 1930's as the "great revolution in muscle physiology." Dr. Mommaerts is going to say more about the revolution Just to refresh your minds, I should like to point out that for a long time it was generally believed that the immediate source of energy for muscle contraction was a type of glycolysis, the breakdown of glycogen to lactic acid. Now, there is no question but that in some phases of the muscle contraction and recovery process, energy from glycolysis and oxidation of carbohydrate is of importance, and is utilized in the over-all contraction process in muscle. However, it became perfectly obvious that there was not a direct link between the energy of those reactions and the mechanical energy of contraction when it was found that muscle contraction could occur in muscles poisoned with iodoacetic acid without lactic acid production; so that we have had to abandon the idea that lactic acid liberation was the direct and immediate source of the energy for contraction

The committee in charge of this symposium has asked Dr. W. F. H. M. Mommaerts, who is Associate Professor of Biochemistry at Western Reserve University and an established investigator of the American Heart Association—I mention this because I think it is of real significance that the American Heart Association, a cosponsor of this session, is engaged in the promotion of scientific work in this field through such activities as the support of the work of Professor Mommaerts—to speak on "The Fundamental Aspects of the Chemistry and Behavior of Contractile Proteins"

9

Three Revolutions in the Physiology of Contraction

WILFRIED F. H. M. MOMMAERTS, Cleveland, Ohio

When at a session like the present a physicochemically oriented physiologist introduces the problem of the molecular nature of contractility, either one of two things may be expected from him. On the one hand, he may give a simple exposition of the fundamental facts (the cardiologic implications of which will then be exposed by the subsequent speaker), presenting well-established results of which the audience knows already but which it likes to be reassured about. Alternatively, he can introduce an element of surprise by presenting an entirely new turn in scientific outlook, upsetting (for the better, *it is* hoped) the picture that had gradually become familiar. I shall bring you some of both.

First, let us become acquainted with the basis of what may be called the "actomyosin-ATP theory."

It is evident that the mechanical activity of contractile tissues such as the heart or skeletal muscle depends on its oxygen consumption or glycolysis. In the late 1920's, it became understood that the relation of these forms of metabolism with mechanical performance is indirect. By poisoning muscles under anaerobic conditions with iodoacetate, both respiration and lactic acid for-

mation could be eliminated. Yet, as Lundsgaard discovered in 1929, contractile activity persists for a considerable period. The chemical reaction covering that activity was found to be a splitting of phosphocreatine (PC). This discovery gave rise to an essay by A. V. Hill on "The Revolution in Muscle Physiology," lactic acid being dethroned from the prominent position given to it by Meyerhof; how it had ever got there, notwithstanding valid experimental objections by Embden, is another problem. For a few years, fission of phosphocreatine was regarded as the cause of contraction, but soon the conclusion was reached in Meyerhof's laboratory that this substance has no independent metabolism in muscle. Instead, the key substance was found to be adenosinetriphosphate, ATP, which can be dephosphorylated to the corresponding diphosphate, ADP, and which in turn can accept a phosphate group from PC, thus effecting breakdown of the latter. In this manner, ATP became regarded as the substance which, through its breakdown, energizes and causes contraction.

During the 1930's it became furthermore understood that, whatever multitude of reactions constitute respiration and glycolysis, they serve but one end: to synthesize ATP from ADP and inorganic phosphate. From the side of metabolism studies, then, knowledge about contractility appeared very satisfactory: Mechanical work is performed at the expense of the chemical energy of ATP, with PC acting as an additional reserve of high-energy phosphate; respiration and glycolysis resynthesize ATP to the extent that it is used up.

What is the nature of the coupling between ATP breakdown and contraction? To answer this fundamental question we have to inquire into the chemical nature and molecular structure of the contractile matter itself. Chemical investigation had shown that the striated fibril consists mainly of a protein preliminarily called myosin, extractible from the minced tissue with strong salt solution. In 1930, Von Muralt and Edsall discovered that flowing solutions of this protein are birefringent, an observation showing that this substance must consist of elongated molecules,

having all the makings of a building-material for the anisotropic bands of the striated fibril.

Having thus identified both the active structure and the presumed energy-supplying substance, the question arises whether an immediate connection between these two entities could be demonstrated. This connection was found in 1939 by Engelhardt and Ljubimova, who discovered that myosin, the structure protein, also is an enzyme, adenosine triphosphatase or ATPase, catalyzing the breakdown of ATP to ADP and phosphate. Several authors in the following years developed the concept that myosin is a contractile enzyme, which captures the energy of the reaction which it catalyzes, using it to perform mechanical work. We have removed ourselves very far now from this concept, but will always recognize what significance the discovery of myosin-ATPase had for what we might call the "Second Revolution in Muscle Physiology."

At this point, A. Szent-Gyorgyi entered the field which he was destined to dominate so markedly during the following years. It was soon found that "myosin" could occur in two forms; the one, more viscous and strongly birefringent, appeared after prolonged extraction of muscular tissue, as opposed to the appearance of the less viscous form after brief extraction. This transformation of one form into another was recognized as due to a combination of myosin itself, isolated by Szent-Györgyi in the crystalline state with a new protein, actin, discovered in the course of this work by Straub. The combination of the two proteins was called actomyosin. Actin too was found to have remarkable molecular properties; it can exist as a globular protein of low molecular weight (G-actin), or in a polymerized, fibrous modification (F-actin). It is the latter form that combines with myosin in a characteristic fashion. Both proteins play an essential role in the architecture of the muscle fibril. Modern electron-microscopic work shows that actin forms filaments which extend throughout the sarcomere with exception of the H and Z lines, *while myosin is arranged in between these filaments in the anisotropic bands.*

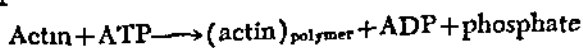
Employing a procedure developed earlier by Weber, Szent-Györgyi spun little actomyosin fibers, and, in a suitable electrolyte medium containing, for example, potassium and magnesium salts, these were found to contract in response to added ATP. This contraction differs from physiologic contraction in that it takes place in all directions and is unable to proceed against an opposing force. These differences, however, are only due to the loose and random internal organization of the thread, which can be improved by special artifices. A very perfect model was later developed by Szent-Gyorgyi without previous dissolution of the structure proteins, by extracting the soluble constituents from muscle-fiber bundles, leaving the bare actomyosin structure intact. This model contracts lengthwise, becoming shorter and thicker, and it develops about the same tension as the muscle from which it is prepared.

Having recognized the performances that actin and myosin can demonstrate, great interest attached itself to the physicochemical properties of these proteins. Weber prepared pure myosin and, at about the same time, we purified both myosin and actin. A short summary of the properties of these materials would read as follows:

myosin: rodshaped molecule, 1500 Å long, 30 Å thick,
G=actin: globular molecule, 40 Å diameter;
F=actin: globular molecules arranged like a string of beads.

These results were obtained with myosin and actin derived from skeletal muscle It is generally held that the corresponding cardiac proteins are similar, although an accurate comparison might well yield some surprises

Further investigation of actin, by Straub and Laki and their collaborators, showed that ATP plays an essential role in the G-F transformation. With pure actin, we could show that this participation is of the nature of a coupled reaction.



$$\text{Actin} + \text{ATP} \longrightarrow (\text{actin})_{\text{polymer}} + \text{ADP} + \text{phosphate}$$

The amounts of reactants participating in this process are such that, if all the actin in 1 gram muscle would polymerize once, it

would utilize just the amount of ATP calculated to be decomposed in one fully activated contraction—0.5 μ mol./Gm. tissue.

But now it appears as if we have too much of the good, "des Guten zuviehl." The one structure protein, myosin, is an enzyme which catalyzes the decomposition of ATP (although we have raised serious doubt as to whether its activity suffices to explain the actually occurring speed of metabolism); the other protein, actin, decomposes that substance stoichiometrically in the right amount. To increase the dilemma, let me bring two more embarassing facts. Engelhardt and Kafiani discovered recently that under suitable circumstances threads of pure myosin can contract with ATP, thus assigning the major role to this protein. At the same time, the highly perfected optical and electromicroscopic work of Huxley and Hanson showed that the myosin in the anisotropic bands of the sarcomere is entirely passive, contraction seemingly existing of a migration of actin filament. It appears as if too much has been discoverd, as if it would be fruitful to take distance from the views to which we became accustomed, and see whether a "Third Revolution in Muscle Physiology" announces itself.

That such an event may be forthcoming is indicated by the results of investigating the chemical changes in living muscle. Some years ago, A. V. Hill insisted upon the necessity of experimentally demonstrating the breakdown of ATP and PC during the course of single muscle twitch. The technical difficulties delayed response to this suggestion, but during the last two years we have been able to carry out such experiments. Of a pair of symmetrical turtle muscles, one is stimulated while the other serves as the control. At a chosen point during the single twitch, activity is suddenly interrupted by precipitous cooling, whereupon both muscles are analyzed with enzymatic-spectrophotometric micromethods. The crucial point is the stoppage of activity; this is achieved by rapid immersion (within 6 milliseconds) into liquid propane cooled to −185°C, with a special apparatus. By means of thermocouple measurements with the aid of an electron-beam oscilloscope, it is found that the rate

of cooling so achieved is indeed very fast, and that the contracting muscle is stopped at the very moment of immersion, the control muscle showing no movement.

Analyses performed at various stages of a single contraction cycle showed that there was no change whatsoever in the amount of ATP, or of precursors that might conceal such breakdown by instantaneous phosphate transfer.

In other words; the splitting of ATP or of PC, for 20 years a cornerstone of our knowledge—seemingly does not take place.

We are unable as yet to evaluate the present situation The demonstration of the ATP-induced contraction of actomyosin and of washed muscle fibers, the connection between ATP and actin, are facts which cannot be ignored: ATP can do something to muscle proteins. Yet, it appears that in living muscle there are entities still closer to the fundamental process, leaving for ATP only a secondary, although undoubtedly still important, role. It may be that in the Szent-Gyorgyi preparation the primary mechanism has already run down, but is recharged by ATP. ATP then remains an important link, but is not the ultimate mechanism.

I have presented the course of our knowledge as a sequence of three "revolutions" More of these may follow. We can be reasonably sure that on future meetings of the World Congress of Cardiology it will again be possible to receive amazement and amusement from the progress of research. It may even be that the day will come when we can be confident that we are progressing in the right direction.

10

*Significance of the Newer Knowledge of Contractile Proteins for Cardiology**

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Within the last 20 years a great deal of interest in cardiology has centered on what may be called applied physiology. The development of new technics has been primarily responsible. Interest of the cardiologist in the sciences which form the basis of cardiology has been of more recent date. The work of biochemists and physicists has given the cardiologist plenty of thought because their studies have focused the spotlight on the physicochemical and metabolic properties of heart muscle. The importance of methods thus developed and the results obtained are only gradually making themselves felt in the thinking of cardiologists. The purpose of this paper is to stress the significance of one aspect of this new approach for cardiology: the recent knowledge of the contractile proteins of heart muscle.

Several methods are used to study the contractile proteins; those which have been most simple and most fruitful to our understanding have been concerned with the creation of "models" of the contractile proteins of heart muscle, in one of

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these procedures heart muscle strips are extracted in water for periods varying from 12 to 15 days [8, 13] All the soluble material, including electrolytes and ATP, are thus removed from the muscle, and protein and lipids remain in the muscle but the cell membrane is destroyed The histologic structure of fresh heart muscle is preserved but the physiologic pattern is greatly altered, for example, the fibers do not respond to electrical stimulation nor do they contract when epinephrine or acetylcholine is added. They only contract with ATP.[15] Thus, they represent models stripped of every activity that can be ascribed to surface or membrane phenomena. Their reaction to load, stretch, etc, is primarily due to substances of which they are basically composed, that is, the contractile proteins. What is the condition of the contractile proteins in these washed strips? In the resting nonextracted muscle, actin and myosin exist side by side in a dissociated form separated by the strongly positive ionic atmosphere due to surrounding K^+ ions ATP is present in the muscle. As soon as the ionic balance of the muscle changes as the wave of excitation sweeps along the membrane, the balance of charges is changed and actin "precipitates" myosin forming the contractile protein, actomyosin, which forms a complex with high energy phosphate ATP.[13] Excitation therefore results in actomyosin formation. During relaxation, the energy which has been expended during the process of contraction is repaid for by release of energy from ATP. Myosin splits ATP and energy is released.

In the extracted muscle which is used as a model, ions, including potassium chloride, sodium, and ATP, have been removed. Actin and myosin are *not* dissociated but actually have formed hysteresis because obviously there are no "protective ions" to keep actin and myosin apart. Actomyosin does not contract because ATP which is necessary for contraction has been removed from the muscle during washing If ATP is added, contraction occurs.

Another method for creating a "model" for the study of contractile proteins was developed by Hayashi.[5] Surface films of actomyosin are prepared which can be compressed into threads

strong enough to lift weight. Using this method, Jane Sands Robb has performed studies on the properties of the contractile proteins of heart muscle that have great clinical significance.[9] The main difference in these two preparations is that in washed heart muscle the histologic structure of heart muscle is preserved. The preparation of Hayashi and Robb has lost any similarity to the original structure of the heart muscle and contains the contractile protein in a "pure" form. The importance of these considerations will become apparent when we discuss the action of digitalis on the contractile proteins of heart muscle.

Let us consider some of the physiologic properties of these models. In studying the washed heart muscle preparation, we can suspend the fibers to a lever and record the isotonic contractions produced by ATP.[15] We can follow the effect of various concentrations of adenosinetriphosphate, we can measure the speed of contraction of extracted heart muscle, and we can finally observe the relationship between the work of the muscle—loads which the muscle has to lift—and the initial length of the muscle preparation. The importance of a sufficient supply of adenosinetriphosphate for muscular contraction is illustrated in experiments in which it was shown that, within certain limits, an increase in adenosinetriphosphate supply results in greater force of contractions.[15] This finding could have been anticipated because ATP appears as the main source of energy directly available for muscular contraction. A minimum amount of 0.2 per cent of ATP is necessary to induce contraction Maximal isometric tensions were recorded with an ATP content of about 1 per cent. Unquestionably, therefore, the supply of ATP determines within certain limits the force of contraction.[15] The speed of contraction of the washed preparation may be determined by measuring the slope of the recorded contraction, because the slope at any given time is proportional to the speed of contraction. It can be seen that the slope decreased as the tension rose. The resemblance between the speed-load relationship of isotonically contracting heart muscle preparation and fresh skeletal muscle is so great as to suggest that the properties of muscle responsible for this relationship are inherent qualities

of the contractile proteins.[15] There we have the first example of the similarity between the properties of the whole heart and those of the contractile proteins.

The work of the extracted heart muscle increases with rising tension up to a maximal value and then decreases as this load is exceeded.[15] The similarity between these findings and those obtained by Frank or Starling on the whole heart is obvious, demonstrating the similarity of the behavior of extracted heart muscle to the whole heart. This similarity extends further. Extracted heart muscle shows a similar length-resting tension relationship to fresh heart muscle, that is, a progressively larger rise in tension with increase in length.[15] Results obtained with the actomyosin thread of Hayashi and of Robb also show some similarity to those obtained on the whole heart.[9] As in the extracted heart muscle, the degree of shortening of the thread is inversely proportional to the weight lifted. In the actomyosin thread, percentage shortening varies with fiber lengths and the smaller the load the higher the contraction As in the washed heart muscle preparation, a critical point is reached where excessive load leads to diminished work output. The similarity here between the two preparations lies in the fact that a change in performance occurs when there is excessive stretch; the concept of "stretch" will play an ever-increasing role in our discussion as we examine the significance of newer knowledge of the contractile proteins.

So far the lesson learned from these preparations has been primarily of importance to the physiologist. The main conclusion reached is that many of the properties of the intact heart can be ascribed to properties of the contractile proteins of heart muscle.

What is the importance of our newer knowledge of the contractile proteins to our understanding of disturbed cardiac function? More specifically, what is the relationship of the contractile elements to the mechanism of myocardial failure? Before going into this discussion, one should consider for a moment the factors which might change the behavior of contractile proteins in heart failure. There is no doubt that increased stretch

occurs in myocardial failure. The degree of cardiac emptying in failure is incomplete because the amount of blood that remains in the heart during isometric relaxation (the residual volume), and consequently the amount of blood which is in the heart during the period of isometric contraction (the diastolic volume), are increased. These volumes, the residual and the diastolic, are a reflection of stretch of the heart muscle; and thus "stretch" may be expressed quantitatively in cubic centimeters or in millimeters of mercury as residual volume or initial tension. *Initial length and initial tension determine the* dynamic and metabolic function of the heart and both initial length and initial tension are increased in heart failure. If there is an increase in the diastolic size of the heart together with a reduction in the stroke volume, cardiac decompensation exists. To use our terminology, myocardial failure is accompanied by excessive stretch of the muscle fibers Excessive stretch of heart muscle leads to loss of elasticity, as Visscher has shown.[7] He demonstrated on the tortoise ventricle that after prolonged increase in intracardiac pressure the diastolic elasticity of the heart muscle is decreased.

What conceivable impact does "stretch" have on the contractile proteins of the heart? How does it lead to decreased elasticity of the heart muscle fibers? We had hoped to get an indirect answer to this question by studying metabolic disturbances occurring in heart muscle. It was reasoned that a change in contractile proteins might result in metabolic alterations. We know now that specific disturbances in metabolism of the heart can be caused by myocardial anoxia [3, 4] It soon became apparent, however, that myocardial failure is not accompanied by specific changes in myocardial metabolism. In heart failure the oxygen consumption of the heart per unit weight is normal and so are the myocardial usage and utilization of carbohydrates, fats, amino acids, and ketone bodies.[1] But the term "normal" is, as usual, misleading. It is true that there are no specific conspicuous alterations in metabolism in failure but the conversion of oxidative energy into mechanical work is at least partially interfered with, thus the conversion of energy derived from all

foodstuffs into active cardiac work is probably also defective.

Are the contractile proteins of the heart responsible for the interruption of the link between the energy intake and output of the heart? Two possibilities suggest themselves. One, that in failure there may be some deficiency in the formation of energy-rich phosphate bonds, the other, that we deal in failure with actual structural physicochemical changes in the contractile proteins of heart muscle. ATP is the primary high-energy phosphate donor in the cell serving as a source of energy for muscular contraction. It was found, as mentioned above, using the glycerol-extracted heart muscle, that the loaded fiber performs a relatively smaller amount of work when the ATP concentration in the surrounding cation solution is diminished below 0.8 per cent.[15] This indicates that heart muscle must have an optimal supply of high-energy phosphate in order to perform maximal work. Certain findings, however, speak against a deficiency in high-energy phosphate in myocardial failure When the glycerol-extracted heart muscle is made to contract isotonically against an increase in load, then the work output first increases. After a certain load is exceeded the work declines.[15] Here, changes in work performance of the fibers are the results of alterations in muscle load only and have little to do with supply of high-energy phosphate. This illustrates that changes in work can occur without alterations in supply of high-energy phosphate. This finding is in line with results of Wollenberger, who has shown that the spontaneously failing heart in the heart-lung preparation retains its normal ATP content and is even richer in phosphocreatine than the nonfailing heart.[16] Apparently here too, it is the utilization and not the generation of phosphate bond energy that is at fault.

Is heart failure accompanied by changes in the contractile proteins? I would not go as far as to say that the results in the washed heart muscle preparation are any proof, but at least they are suggestive. It could be shown that when the extracting fibers were submitted to periods of stretch lasting from 2 to 3 weeks, they performed more work than did the fibers which were not stretched during the extraction period. This suggests that

stretch is an important factor in determining the work of the heart regardless of whether stretch signifies increased initial length or increased initial tension. It is therefore quite conceivable that increased stretch changes the contractile proteins and that it is this change which leads to heart failure. It is of interest in this connection that the phosphorylating sarcosomes of cardiac muscle are located contiguous to the A bands of the myofibrils, indicating the close relationship between the site of energy production and the biostructures of energy utilization.[10]

Finally, mention should be made of the role of contractile proteins in the action of digitalis. There is little doubt now that the cardiac glycosides act on heart muscle directly Is this action production within the cell, is it on the contractile proteins, or is it on the membrane activity of the muscle cell? Digitalis has little effect on the myocardial extraction of carbohydrate and noncarbohydrate substrates from the coronary blood.[2] Friedman and St. George found that digitoxin failed to combine with the mitochondrial components of the cell but was found within the component of the cell containing actin, myosin, and ATP;[11] these findings suggest that digitoxin may exert its effect directly on the contractile element. The results of Robb point the same way.[9] Cardiac glycosides make it possible for actomyosin threads to undergo greater shortening and shorten more rapidly. Workers in Sweden came to the conclusion that the basic effect of digitalis may be on polymerization of actin,[8] they also believe that cardiac glycosides have a direct influence on the contractile proteins by influencing the ionic balance in the environment of the muscle fiber. So far, the evidence points toward a direct action of digitalis on the contractile proteins.

All would be well were it not for the fact that digitalis has no effect on the washed heart muscle preparation which we have considered as a model of the contractile protein[13] Specifically, Cedilanid has no effect on the work-tension relationship, the speed of contraction, or the length—resting-tension relationship of extracted heart muscle preparation. How can we reconcile these results with those of Robb? There may be several reasons for the difference. Robb's fibers represent relatively pure acto-

myosin. The washed heart muscle strips contain sarcolemma and its histologic structure is well preserved In Robb's experiments the glycoside was added directly to the actomyosin solution. In experiments on washed heart muscle it was either injected into the animal or added to the surrounding bath. Finally, periods of extraction in water and glycerol for several days may have broken the link between the glycoside and the contractile substances. However, the final reason for the discrepancy is not know

If there is as yet no complete agreement on action of digitalis on the contractile proteins, there is no question that digitalis acts on the membrane activity of heart muscle. This is illustrated when, using the microelectrode technic of Gerard, membrane action potentials are led off single heart muscle fibers.[12, 17] There is loss of overshoot and diminution in the amplitude of depolarization and shortening of repolarization. The strong affinity of the glycosides for heart muscle is well known. This is illustrated in experiments in which it is shown that membrane action potentials of fibers that have been bathed in Tyrode solution containing Cedilanid maintain their digitalis effect when placed in Cedilanid-free Tyrode solution.[12] Theoretically, the glycoside could have remained attached to the membrane or it could have penetrated the interior of the cell combining with the contractile proteins. We attempted to find an answer to this question by destroying the membrane through water extraction. When muscle is first digitalized in Tyrode solution and then extracted in water for 2-6 minutes until mechanical electrical activity ceases and the muscle is then brought into contact with Cedilanid-free Tyrode solution, action potentials reappear demonstrating digitalis effect on their first reappearance.[12] If it is assumed that water extraction destroys the membrane temporarily, then the glycoside must have become fixed to the interior of the cell, to the proteins. On the other hand, the glycoside may have been bound to the membrane itself which may have only temporarily lost its ability of depolarization and repolarization, presumably due to change in permeability to sodium and potassium.

When membrane activity is initially abolished by soaking the muscle strip in water and the fibers are then exposed to Tyrode solution containing Cedilanid, the reappearing action potential bears all the marks of glycoside activity.[12] Here, too, however, it is difficult to decide whether or not Cedilanid has been bound to the contractile proteins. The fact that the membrane action potential reappeared at all makes it unlikely that the membrane had been completely destroyed during the process of extraction.

The results of these experiments, therefore, are inconclusive; we still do not know whether digitalis acts on the contractile proteins alone or on the membrane exclusively or on both proteins and membrane alike. However, we feel that on reviewing the subject, we are justified in saying that the effect of cardiac glycosides on contractile proteins and on membrane activity is probably inseparable; most likely the glycosides act through a common denominator. We have seen how in the resting muscle the myosin particles are kept straight and in solution by being surrounded by an atmosphere of positive potassium ions We also have heard of the profound changes in the ionic equilibrium between the cell and its surroundings occurring during the excitation process As a matter of fact, ions have to be mentioned at every turn, as Szent-Györgyi says, when muscular contraction and relaxation are discussed.[13] It is the intracellular ionic atmosphere that determines the charges, the balance of repulsive and attractive forces, the trend of actin and myosin to part or to get together, and last but not least, the ionic atmosphere decides also the tension developed in contraction The electrical manifestations of excitations are, of course, an expression of ionic changes. The membrane not only produces, propagates, and transmits excitation, but at the same time it regulates, by its specific permeability, the intracellular ionic atmosphere which decides the behavior of the contractile proteins.

For this reason, membrane activity and the physicochemical pattern of contractile proteins are inseparable, and what we observe with digitalis is the effect of ions on both contractile proteins and on electrical activity, the latter being only a manifectation. Szent-Györgyi found that muscular contraction, which

had been weak when the heart was perfused with Ringer's solution, improved when serum was substituted.[14] This effect of serum can be ascribed to the presence of some sterols. Digitalis had a similar effect. Szent-Györgyi reasoned that when the heart shows diminished contraction under experimental conditions, for example, when it was perfused with Ringer's solution, it lacks the regulating sterol which helps to keep the potassium out of the cell or is merely unable to bind the sterols. We have, therefore, reason to believe that the effect of cardiac glycosides on membrane activity and on contractile proteins are the result both of ions and of their different distribution across the cell membrane.

Let me summarize the significance of the newer knowledge of contractile proteins to cardiology. In the first place there is little doubt that many of the features of the whole heart *in vivo* are the result of inherent properties of the contractile proteins. There is also good evidence that many of the features of myocardial failure are due to inherent disturbances within the contractile proteins of the heart muscle. These disturbances may be the result of disorientation in the physicochemical structure of actomyosin or of ionic changes affecting the contractile proteins through changes in membrane permeability. Finally, the action of digitalis is probably also related to properties of the contractile proteins mediated through ionic changes within the cell.

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